



## PHD

### **Sexual conflict, ecology and breeding systems in shorebirds: phylogenetic analyses**

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**Sexual conflict, ecology and breeding systems in shorebirds:  
phylogenetic analyses**

Gavin Huw Thomas

A thesis submitted for the degree of Doctor of Philosophy

University of Bath

Department of Biology and Biochemistry

November 2004

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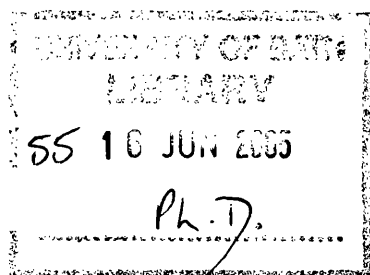
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## Summary

Males and females often have divergent interests over reproduction resulting in inter-sexual conflict over mating and parental care. I use phylogenetic comparative analyses to investigate the conflict over care in shorebirds (sandpipers, plovers, gulls and allies; 350 species). First, I reconstructed the phylogenetic relationships of shorebirds using molecular sequence data using parsimony, maximum likelihood and Bayesian analyses. Using a supertree approach, I also produced the first complete phylogeny of shorebirds. Second, I used the phylogenetic framework of the supertree to ask four questions: (i) Do the demands of the young constrain rates of phenotypic evolution. Using a novel phylogenetic technique, I show that the outcomes of sexual conflict are more diverse in taxa that have precocial offspring than those with semiprecocial taxa. (ii) Does sexual conflict influence the mating behaviour of shorebirds; directional comparative analyses indicate that parental care and social mating systems of shorebirds evolved in a feedback loop consistent with predictions of sexual conflict theory. (iii) Why are some shorebird lineages more species rich than others? I found that the different mating optima of the sexes are associated with species richness. (iv) Why are some shorebird lineages more prone to decline than others? I found that continental migratory routes and to a lesser extent conflict over mating optima are associated with population trends. In conclusion, my work suggests that the intensity of parental conflict varies among shorebird taxa, and that this conflict has implications on evolutionary rates, speciation and extinctions. The research presented herein has advanced our understanding of the impacts of sexual conflict over care, although important questions remain to be answered; for example, we do not yet know whether the causes and consequences of sexual conflict are consistent amongst birds, nor how human populations influence population trends in shorebirds.

## Acknowledgements

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**Chapter 1.** *Sexual conflict, ecology and breeding systems in shorebirds: phylogenetic analyses*

Gavin H. Thomas



## 1. INTRODUCTION

Males and females often have divergent interests over reproduction. It is in the interest of both sexes to maximise the number of viable offspring, yet there are different pathways to this goal that are fraught with intersexual conflict (Chapman *et al.* 2003). At the centre of this conflict is the hugely different investment in the gametes by males and females (anisogamy; Bateman 1948). The size of male sperm in comparison to the female egg is miniscule in many animals. By producing fewer but substantially larger gametes, females limit the reproductive rate in sexually reproducing species. Males, in contrast, produce vast numbers of small gametes. Consequently, males are expected to benefit from mating with as many females as possible and, where mating opportunities are limited, to compete for mates (Bateman 1948; Andersson 1994). The divergent interests of males and females over reproductive optima have many important implications from the genomic to the phylogenetic level.

The interests of the sexes may also differ over care provisioning (Parker 1979; Lessells 1999; Houston *et al.* 2005). Parental care is costly for the care-giver because it often reduces future survival and reproduction (Clutton-Brock 1991). Each parent will benefit by desertion if the costs of reduced offspring survival are less than the costs of reduction in their own survival or the loss of reproductive opportunities in the future. Offspring survival is likely to be enhanced if at least one parent provides care, and it is in the best interests of each parent if the other provisions for the young (Lessells 1999; Houston *et al.* 2005). The outcomes of sexual conflict over parental care are therefore likely to be pivotal in determining the social mating system of a species, and may also impinge on macroevolutionary processes such as speciation and extinction.

The objectives of my PhD were to investigate causes and consequences of sexual conflict over care using a phylogenetic approach. Typically, biologists use either experiments (Rice 1996; Arnqvist & Rowe 1995; Holland & Rice 1999; Royle *et al.* 2002) or mathematical models (McNamara *et al.* 2000; Gavrillets *et al.* 2001; Parker *et al.* 2002) for analysing sexual conflict. I took a different path, and used phylogenetic comparative methods to investigate the intensity of sexual conflict over care, and the implications of conflict among the avian order Charadriiformes (shorebirds, gulls, and alcids; 350 species).

Specifically, I asked:

- *What are the phylogenetic relationships between shorebird taxa?*
- *Do the demands of the young constrain the expression of sexual conflict?*
- *Does sexual conflict drive the evolution of breeding systems?*
- *Why are some shorebird lineages more species rich than others?*
- *Why are some shorebirds in decline but others stable or increasing?*

For this work I used a range of phylogenetic comparative methods. Below, I outline the theoretical background behind the thesis, and then discuss why shorebirds are an ideal group to address these questions. I then discuss the specific objectives and novel results of each chapter. Finally, I highlight potential avenues for future studies of sexual conflict over care.

## **2. BACKGROUND: RUNAWAY OR CHASE-AWAY?**

### **(a) *Sexual conflict and sexual selection***

*“...the males have acquired their present structure, not from being better fitted to survive in the struggle for existence, but from having gained an advantage over other males, and from having transmitted this advantage to their male offspring alone, sexual selection must here have come into action.”*

*Charles Darwin, 1871 (p210)*

Darwin (1871) observed that in many species, males possess elaborate ornaments, colouration, displays, songs, and fighting apparatus that he supposed would surely reduce their chances of survival. Such traits seem to act in direct opposition to natural selection in which traits should be selected to maximise survival. This juxtaposition stimulated Darwin to develop his “other theory” of sexual selection. Why do apparently costly traits persist if they do not enhance survival?

Darwin (1871) proposed two routes to the evolution of sex differences. First, secondary sexual characters that enhance fighting ability (e.g. horns, antlers and clubs) are associated with male-male competition for mates. Second, the apparently inexplicable elaborate plumes, ornaments, displays, songs, and colouration of males in many species are the result of the cumulative effects of female choice. Fisher (1930) argued that female choice of the most attractive mates can result in more attractive male offspring which themselves have a higher chance of being chosen. Thus, there is a mutualistic coevolution of “attractiveness” traits and female choice of such traits. Secondary sexual characters can evolve rapidly in a positive feedback runaway process (Fisher 1930) as males that are more attractive are selected by females who perpetually raise the bar.

Recently, an opposing form of sexual selection has challenged the traditional mutualistic view of male-female coevolution. Conflict between males and females arises wherever one sex invests more in reproduction than the other sex (Trivers 1972). Since females generally invest considerably more in the gametes than the males (anisogamy; Bateman 1948), males are expected to benefit from multiple matings whereas females, in general, would not (Bateman 1948; Trivers 1972). However, sexually selected characteristics that are advantageous to males may not be advantageous to females and may even be deleterious. Thus, sexually selected characteristics may evolve along antagonistic, rather than mutualistic, trajectories (Parker 1979; Chapman *et al.* 2003). Adaptations that benefit one sex at the expense of the other should drive counter-adaptations to avoid exploitation by the other sex (Trivers 1972). This battle of the sexes was termed sexual conflict by Parker (1979). As with Fishers run-away selection, sexual conflict may drive the rapid divergence of the characteristics under selection except that the process is in the form of a chase-away arms race (Rice & Holland 1997; Holland & Rice 1998). There is a growing body of evidence that sexual conflict is a pervasive evolutionary force and is likely to drive antagonistic coevolution of a range of traits including reproductive anatomy, physiology, and behaviour (e.g. Eberhard 2004; Arnqvist & Rowe 2002; Pitnick *et al.* 2001).

Males and females may also disagree over care provisioning. Parental care conveys costs to the caregiver by means of reduced future survival and reproduction (Clutton-Brock 1991). The decision whether to provide care or not, and the amount of care provided, depends on the potential future costs of reduced mating opportunities balanced

against the immediate costs associated with parental care (Balshine *et al.* 2002). The basic principle of sexual conflict over care is that each parent should prefer the other to do the hard work of raising the offspring in order to maximise their own fitness (Lessells 1999; Houston *et al.* 2005). Thus, there are three viable outcomes of sexual conflict over care (assuming that parental care enhances offspring survival in a multiple-breeding animal). First, both parents cooperate and provide care for the offspring. This may be a necessity if the demands of the young are high, or if future mating opportunities of both sexes are limited. Second, the male parent deserts and the offspring are raised predominantly by the female parent. Third, the female parent deserts and the offspring are raised predominantly by the male parent. The latter two outcomes are likely if the demands of the young are low, or if future mating opportunities are high for the male or the female respectively. Consequently, it is always in the best interests of the male parent for the female to provide care, whereas it is in the interest of the female parent to have the male provide care. Parental behaviour that reduces the potential for exploitation by the opposite sex should be favoured over behaviours that are optimal only when all parties cooperate (Maynard-Smith 1977; Székely *et al.* 1996; Houston *et al.* 2005).

#### ***(b) Sexual conflict and the evolution of mating systems***

Classical mating system theory states that the social mating system of a species is a consequence of the ability of one sex to monopolize members of the opposite sex (Emlen & Oring 1977). Monopoly may be achieved indirectly, for instance by controlling essential resources, or by directly defending mates. In socially monogamous systems, neither sex has control over more than one mate. In socially polygynous systems, males monopolize females, whereas in socially polyandrous systems females monopolize males. According to this view, resources determine the distribution of females, and females determine the distribution of males (Davies 1991). Sexual conflict theory challenges the ecological view of the evolution of animal mating systems. The resolution of sexual conflict over care (which, if any, parent is more likely to desert the brood, and the effort that each parent provides for the young; Houston & Davies 1985) is likely to depend on mating opportunities. The benefits of desertion depend on the availability of mates which itself is a product of the population sex ratio and mating system (Reynolds 1996; Székely & Cuthill 2000). Hence, the optimal mating system and parental care strategy are tied into a perpetual feedback loop in which mating and

parental behaviours influence, and are influenced by, mating opportunities (Székely *et al.* 2000a). The mating strategy of an individual depends on its parental care strategy as well as the mating and care strategies of all other adults in the population. Thus, breeding systems (incorporating mating systems and parental care; Reynolds 1996) do not necessarily depend on ecology, and may diverge even in an ecologically stable setting (McNamara *et al.* 2000).

Why does sexual conflict appear to be more intense in some species than in others? In species that display lifelong monogamy with random mating there are no conflicts of interest in reproduction since the maximum fitness is achieved by cooperating with the mate (Rice 2000; Houston *et al.* 2005). In all other cases, there exists the potential for sexual conflict. As discussed above, mating opportunities are likely to limit the outcomes of sexual conflict; however this may be a highly dynamic relationship. Direct constraints on the expression of sexual conflict over care may arise as a consequence of the demands of the young. Where the offspring are needy, for example by being underdeveloped, or by living in a harsh environment, the options for care are likely to be limited and it is expected that both parents must provide care.

Of course, the demands of the young may themselves be dynamic. For instance, in a changeable environment (variation in climate, food availability, predation risk) the levels of care required by the young will likely change from year to year, or even within a single breeding season. Predictions on the outcomes of sexual conflict based on such factors are therefore extremely difficult. However, the physiological, morphological and behavioural state of the young at birth or hatching (developmental mode) is highly conservative across species with most variation at the family level or above (Starck & Ricklefs 1998). In some species, the offspring are entirely dependent on the parents (altricial species), whereas in others they are at least partly independent (precocial species). Developmental mode is expected to constrain the expression of sexual conflict over care. In species with altricial offspring the potential for evolution of characteristics associated with sexual conflict will be limited. In contrast, species with precocial offspring are likely to be associated with a range of sexual conflict outcomes. The rate of evolution of traits associated with sexual conflict is therefore likely to be high in species with precocial offspring.

***(c) Macroevolutionary consequences of sexual selection and sexual conflict***

The influence of the Fisherian runaway process has long been recognised as a potential driving force in speciation (Lande 1981). Rapid divergence of sexually selected traits, particularly in allopatric populations, is likely to lead to the formation of reproductive barriers and consequently to speciation. This is equally true of sexual conflict. Traits associated with sexual conflict (reproductive anatomy, physiology, and behaviour) may contribute to pre- and post-zygotic reproductive isolation. As sexually antagonistic coevolution proceeds in isolated populations, the divergence in these traits is likely to promote speciation (Rice 2000). This has received recent theoretical and experimental support (Rice & Holland 1997; Parker & Partridge 1998; Arnqvist *et al.* 2000).

Whilst sexual selection and sexual conflict may promote the birth of a species, they may also bring about species' death. Sexual selection pressure on species to evolve traits that enhance mating opportunities may compromise benefits normally accrued through natural selection (see Andersson 1994). Species that undergo more intense sexual selection are likely to be less able to adapt to a changing environment and thus are more prone to population extinction (Tanaka 1996). The focus to date has been on the influence of sexual selection, yet it is evident that sexual conflict is equally likely to compromise benefits established through natural selection because the ability to adapt to environmental change will also be compromised in individuals and species embroiled in an arms race between the sexes. Indeed, in a comparative study of birds Owens & Bennett (1997) found that increased post-hatching care in one sex is associated with increased mortality in that sex. Liker & Székely (submitted) expanded this conclusion and found that both parental care and mating competition are costly for survival, although these costs were only detectable in males.

Taken together, sexual selection and sexual conflict are pervasive evolutionary forces that are likely to impact on a range of levels from interactions between males and females over care to macroevolutionary trends. Patterns of parental care and social mating system are likely to be strongly influenced by both sexual conflict and sexual selection. Furthermore, variations in species richness and extinction risk may have arisen through variation in the intensity of sexual selection and sexual conflict across species.

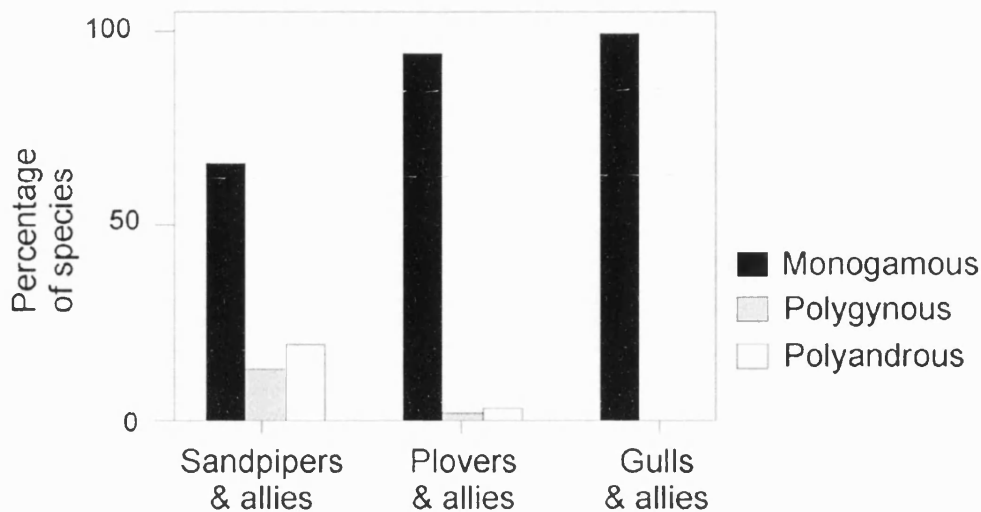
### 3. SHOREBIRDS, GULLS, AND ALCIDS

The 350 species of shorebirds, gulls, alcids and allies that comprise the diverse avian order Charadriiformes (hereafter shorebirds) have a long and venerable history of interest amongst evolutionary biologists and ornithologists alike. Few groups of birds can match the rich diversity of shorebirds. As an order, they are found on every continent, and breed in some of the most inhospitable places on Earth ranging from the high Arctic (e.g. many sandpipers, especially the genus *Calidris*, skuas and jaegers) and Antarctic (sheathbills), to desert and semi-desert (e.g. pratincoles and coursers); others prefer the relative luxury of northern temperate zones (representatives of most shorebird families) and the tropics (e.g. jacanas, painted-snipe). Across this range they are not limited to the coast or to the edge of lakes and rivers as their name suggests; many species breed inland, in habitats ranging from streams in the Himalayas (e.g. ibisbill *Ibidorhyncha struthersii*) to arable farmland (e.g. northern lapwing *Vanellus vanellus*). Perhaps the most striking feature of many shorebirds is their spectacular migratory journeys. Red knots (*Calidris canutus*) travel over 18,000 miles per year, some bar-tailed godwits (*Limosa lapponica*) fly non-stop from Alaska to New Zealand, whilst the Arctic tern (*Sterna paradisaea*) probably migrates further than any other bird species covering 22,000 miles per year from the Arctic to the Antarctic and back again. The migratory behaviour of many shorebirds makes them potentially susceptible to habitat loss on the wintering and breeding grounds as well as at stopover sites en route. It is therefore particularly concerning to note that almost half of the world's known shorebird populations are in decline (International Wader Study Group 2003) as this may indicate a more widespread problem.

#### (a) *Shorebird mating systems*

The range of social mating systems of shorebirds is unsurpassed in any other avian order (Pitelka *et al.* 1974; Erckmann 1983), suggesting a continuum in the intensity of sexual selection; indeed, Darwin (1871) referred to several species of shorebird to illustrate his theory of sexual selection. In contrast, rates of extra pair paternity are uniformly low (0-8% of broods; Griffith *et al.* 2002,  $n = 14$  species; Blomqvist *et al.* 2002,  $n = 3$  species). Species range from full social polygyny (e.g. territorial polygyny: pectoral sandpiper *Calidris melanotos*; lekking: ruff *Philomachus pugnax*, buff-breasted sandpiper *Tryngites subruficollis*), through social monogamy (oystercatchers, many plovers, gulls,

alcids), to classical polyandry (e.g. great painted-snipe *Rostratula benghalensis*, African jacana *Actophilornis africanus*). However, the distribution of polygamous species is not even (fig. 1). The gulls and allies are almost exclusively monogamous with biparental care.



**Figure 1.** The distribution of social mating system across the three major shorebird clades (11 polygynous species, 199 monogamous species, 17 polyandrous species). Note that data are not available for some species.

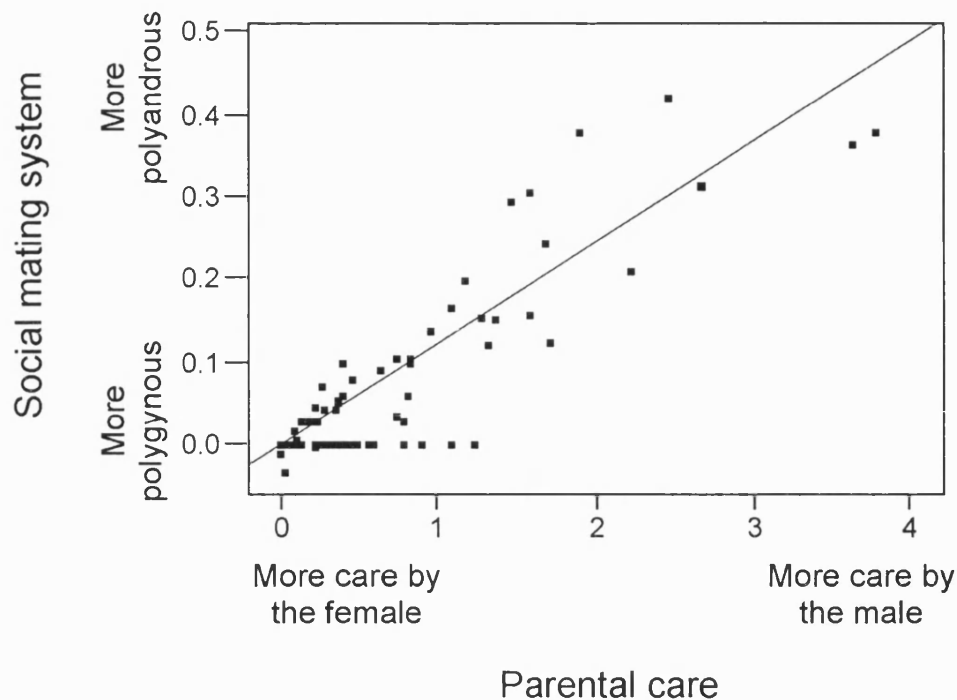
Overall, the proportion of polyandrous shorebirds is marginally higher than that of birds as a whole (around 5% in shorebirds and probably less than 5% of all bird species; Bennett & Owens 2002). Yet, amongst the sandpipers and allies, 10-15% of known species are polyandrous (fig. 1). This is all the more remarkable when one considers that the distribution of polyandrous shorebirds does not appear to be ecologically or geographically limited. There are polyandrous shorebirds from the tropics (e.g. pheasant-tailed jacana *Hydrophasianus chirurgus* and bronzed-winged jacana *Metopidius indicus*) and northern temperate zones (Wilson's phalarope *Steganopus tricolor*) to the high arctic (e.g. red phalarope *Phalaropus fulicaria* and red-necked phalarope *Phalaropus lobatus*). In a study across all birds, Owens (2002) demonstrated that female-only care (associated with polygyny) occurs at high nesting density whereas male-only care (associated with polyandry) occurs at low nesting density. However, much of the variation in parental care and social mating system remains to be explained



and it is likely that social interactions, in addition to ecological correlates, could provide new insight.

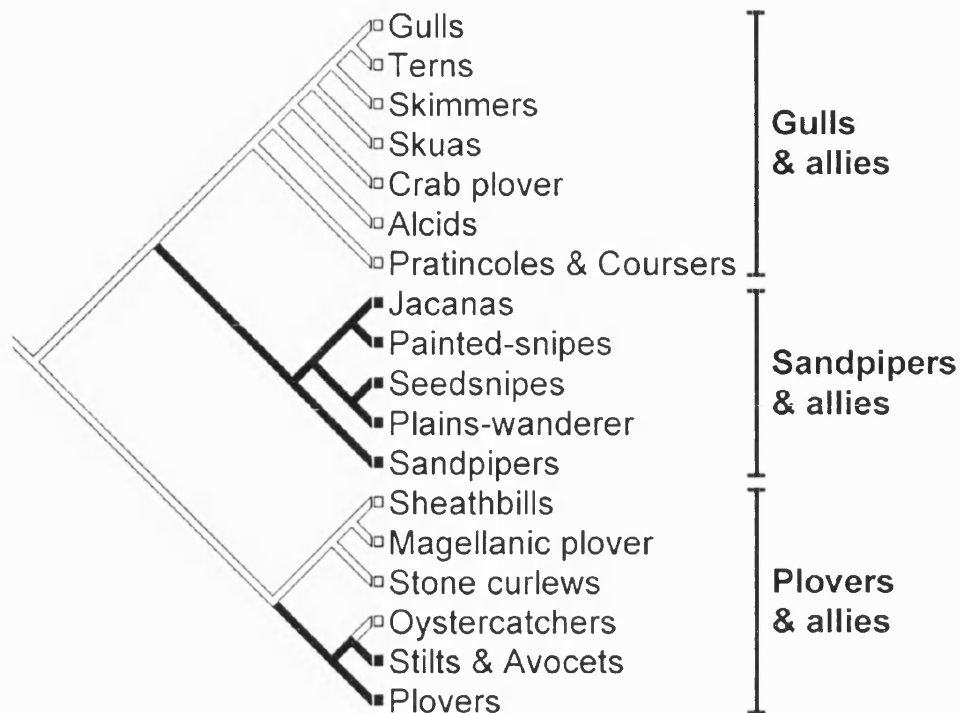
**(b) Sexual conflict in shorebirds**

The range of mating systems observed amongst shorebirds is a clear indicator of varying levels of sexual selection and this is supported by comparable variation in other indicators of sexual selection including sexual size dimorphism (Jehl & Murray 1986; Székely *et al.* 2000b) and male display behaviour (Figuerola 1999; Székely *et al.* 2004). However, it may also reflect sexual conflict over parental care. The range of parental care in shorebirds is as remarkable as the diversity of mating systems (Pitelka *et al.* 1974; Erckmann 1983; Székely & Reynolds 1995). Shorebirds range from female-only care (e.g. jack snipe *Lymnocyptes minimus*, sharp-tailed sandpiper *Calidris acuminata*) to male-only care (e.g. plains wanderer *Pedionomus torquatus*, dotterel *Eudromias morinellus*). Even in monogamous species, the duration of care provided by the sexes differs and it is frequently the male that works harder (e.g. dunlin *Calidris alpina*, semipalmated plover *Charadrius semipalmatus*). A previous study has shown that evolutionary increases in the duration of care of one sex are associated with decreases in the duration of care of the other sex (Reynolds & Székely 1997) indicating that if one parent deserts the brood, the other parent must compensate. The trade-off is highly suggestive of conflict between the parents over care. Furthermore, there is a tight correlation between the difference between the sexes in the duration of care and social mating system (reduced major axis through the origin:  $b = 0.122$ ,  $P < 0.001$ ,  $n = 175$  phylogenetically independent contrasts; fig. 2). However, it is not clear whether changes in parental care drive changes in social mating or *vice versa*.



**Figure 2.** The relationship between parental care and social mating systems in shorebirds. The independent axis shows phylogenetically independent contrasts in parental care bias between the sexes (i.e. duration of male care – duration of female care). The dependent axis shows phylogenetically independent contrasts in social mating system (polygyny → monogamy → polyandry). Note that social mating system refers to the type of pair-bond, whereas parental care refers to the duration of care.

It is notable that parental care, like social mating system, is most variable amongst the sandpipers and allies, and is limited to biparental care in the gulls and allies, whereas it is intermediate in the plovers and allies. This may be attributed to the distribution of precocial versus semiprecocial species (fig. 3). All sandpipers have precocial offspring whereas all gulls have semiprecocial offspring. The plover clade, which is intermediate in the range of parental care and social mating system, contains a mixture of precocial (e.g. all true plovers [*Charadrius spp.*], lapwings, stilts and avocets) and semiprecocial (e.g. all stone curlews and oystercatchers) species.



**Figure 3.** Reconstruction of ancestral developmental modes in shorebirds using maximum parsimony (Maddison & Maddison 2001). Black lines indicate precocial young; white lines indicate semiprecocial young. Amongst the semiprecocial species, monogamy and biparental care is prevalent (see Chapter 4). In contrast, male- and female-only care, and both polygynous and polyandrous mating systems are notable amongst species with precocial young.

It is clear that shorebirds are an exceptionally diverse group and that, perhaps more than any other avian order, they are likely to express a wide continuum of sexual conflict over parental care. Taken together, the diversity of shorebirds, particularly with respect to mating and parental behaviour, coupled with their geographic and ecological variety make them an ideal group to investigate the macroecological and macroevolutionary consequences of sexual conflict and sexual selection.

#### 4. SEXUAL CONFLICT, ECOLOGY, AND BREEDING SYSTEMS IN SHOREBIRDS

##### *(a) Shorebird phylogeny*

*“...the phylogenetic dog can really bite if ignored.”*

*Mark Pagel, 1997 (p333)*

Biologists have been using cross species comparative studies to investigate evolutionary hypotheses ever since Darwin (1859), and it is well established that closely related species tend to be more similar to one another than more distant relatives (Felsenstein 1985; Harvey & Pagel 1991). However, it is only in the last two decades that the effects of phylogeny have regularly been explicitly accounted for in comparative analyses (for extensive examples see Bennett & Owens 2002). Indeed, phylogenetic approaches to cross-species studies are now regarded as the “gold-standard” (Huelsenbeck *et al.* 2000). Thus, to understand broad-scale evolutionary patterns amongst shorebirds requires that a phylogenetic comparative approach be employed, and this, in turn, requires a phylogeny.

Shorebird classification and taxonomy have a long history dating back to Linnaeus (1758), who identified the close affinities between sandpipers, avocets, oystercatchers, and plovers. However, it was over 200 years before Joseph Strauch (1978) made the first serious attempt to resolve the phylogeny of the group using morphological characters. Strauch’s (1978) study revealed three main lineages; the alcids, the sandpipers and allies, and the plovers and allies (including gulls), however, his analyses did not separate out the relationships between these lineages. Strauch’s (1978) data has since been revised and reanalysed on at least four occasions (Mickeyvich & Parenti 1980; Björklund 1994; Chu 1995; Dove 2000). In these latter analyses, the three main lineages remained intact with the alcids as the basal lineage. However, molecules and morphology disagree over the deep nodes of the shorebird phylogeny. Sibley & Ahlquist (1990), using DNA-DNA hybridisation, also found three major lineages but these were not consistent with hypotheses based on morphology. They resolved sister groups consisting of the gulls, alcids and allies, together with the plovers and allies, and a basal lineage of sandpipers and allies. Broad studies of shorebirds using molecular data and modern reconstruction techniques have been conspicuous by their absence.

Furthermore, there has been no attempt to resolve the phylogeny of all extant shorebirds to date.

Choosing the best phylogeny for a comparative study of shorebirds presents a major dilemma. The disagreement amongst existing shorebird phylogenies means that most (and probably all) are likely to contain error. Moreover, all are incomplete with regard to the extant members of the order. Of course, the one true phylogeny is the unobtainable (and unknowable) goal, but in the absence of such omniscience, the choice between competing hypotheses becomes somewhat subjective. It is therefore desirable first, to resolve the conflict over the basal nodes of the shorebird tree, and second, to reconstruct the relationships of the entire group. I address these issues in Chapters 2 and 3.

First, I analysed sequences of the mitochondrial cytochrome-*b* gene from 86 shorebird species representing 11 families with Bayesian, parsimony, minimum evolution, and quartet puzzling methods. The results challenge both morphological and previous molecular hypotheses (Chapter 2). I found strong support for four major lineages consisting of gulls, alcids, plovers, and sandpipers, respectively. However, the broad structure of the phylogeny differed substantially from previous hypotheses of shorebird phylogeny in placing the plovers at the base of the tree below the sandpipers in a pectinate sequence towards a large clade of gulls and alcids. These relationships have been corroborated by recent molecular studies of the nuclear genome (Ericson *et al.* 2003; Paton *et al.* 2003) and indicate that early morphological studies may have been misled by the highly derived morphology of alcids.

Second, I constructed a supertree of shorebirds including estimates of branch lengths in units of time (Chapter 3). Supertree methods combine existing phylogenetic hypotheses into a single analysis and thus provide an efficient means by which to estimate phylogenetic relationships on a large scale. This is the first phylogeny of shorebirds to include all extant members of the group. Furthermore, the topology is largely consistent with recent molecular studies. Taken together, the taxonomic coverage and consistency with molecular data make the supertree an ideal phylogeny for use in comparative studies. Thus, the supertree provides the basic phylogenetic framework for all

subsequent analyses in this thesis, and has been used in other published research (Bokony *et al.* 2003; Thomas *et al.* 2003).

**(b) *The demands of the young***

Explicit biological explanations for phylogenetic constraint have been identified yet ancient changes in ecology and life-histories may have played an important role in determining the patterns of diversity amongst birds (Owens & Bennett 1997; Bennett & Owens 2002). Temrin & Tullberg (1995) demonstrated that polygamous mating systems and short pair bonds have arisen more frequently in taxa with precocial offspring than in taxa with altricial offspring. However, the full influence of developmental mode on sexual conflict, life-histories, and ecology was not explored. Moreover, Temrin & Tullberg's (1995) study did not separate the effects of behavioural differences in offspring development from the effects of morphological and physiological differences.

I co-developed a novel maximum likelihood method to compare rates of phenotypic evolution between groups of species (Chapter 4). I used this method to compare precocial and semiprecocial shorebirds to test whether developmental mode limits the expression of sexual conflict, and the diversity of life-history and ecology amongst species (Chapter 4). The results show that rates of evolution of social mating system and parental care are higher amongst species with precocial young than those with semiprecocial young. This is the first comparative study (to my knowledge) that explicitly demonstrates that offspring feeding behaviour, rather than morphology and physiology, limits parental and mating behaviour. In addition, I extended on the conclusions of Temrin & Tullberg (1995) by demonstrating that developmental mode may also limit the expression of sexually selected traits, notably sexual size dimorphism in body mass. Furthermore, ecological and life-history traits were also related to developmental mode. Body mass and wing length have diverged more rapidly amongst semiprecocial taxa than precocial taxa, but adult mortality shows the opposite trend. In addition, precocial species have diverged more rapidly in habitat quality and in bill length dimorphism.

**(c) *Sexual conflict and shorebird breeding systems***

Close associations between social mating systems and parental care (fig. 2) are well known (Ligon 1999; Bennett & Owens 2002), yet we know relatively little about the

nature of this relationship. On the one hand, classic parental investment theory predicts that parental care decisions determine the type of social mating system; for instance, polygamy should arise only after transitions to uniparental care. On the other hand, sexual conflict theory predicts that sexual selection (the competition among both males and females for mates), mating opportunities and parental behaviour are interrelated, and that there should be a feedback loop between mating competition and parental care (Houston *et al.* 2005). Furthermore, although I show in Chapter 4 that the range of parental care and social mating strategies is linked to developmental mode, it is not clear what drives this relationship. Finally, we do not know whether the trade-off over parental care by males and females (Reynolds & Székely 1997) is driven by the male or by the female.

Using a directional phylogenetic comparative method (Pagel 1994) I found that both parental care and mate competition (i.e. social mating systems) most likely responded to changes in developmental mode (Chapter 5). Evolutionary transitions towards more precocial offspring preceded transitions towards reduced care and social polygamy. Furthermore, I found that changes in care and mating competition respond to one another, as predicted by sexual conflict theory. Finally, changes in male care and female care respond to one another, and neither sex consistently drives this relationship. Taken together, these results are more consistent with sexual conflict theory than with parental investment theory. The results presented in Chapter 5 represent the first explicit (to my knowledge) comparative test of sexual conflict over parental care.

#### **(d) *Species richness***

Why are some families more species rich than others? Some shorebird families contain only a single species (e.g. Dromadidae, Pedionomidae), whilst others contain many (Scolopacidae, 86 species; Charadriidae, 65 species; Laridae, 51 species). Previous comparative studies across a wide range of taxa have failed to provide consistent explanations for variation in species richness. For example, amongst birds, more intense sexual selection has been associated with increased species richness in some studies (Owens *et al.* 1999; Mitra *et al.* 1996), whereas others found no relationship (Morrow *et al.* 2003). No previous study has addressed speciation in shorebirds.

In Chapter 6, I show that the variation in species richness amongst shorebirds is significantly different than expected by chance alone. This variation could not be adequately explained by differences in sexual selection, sexual conflict, niche availability or life-histories across all shorebirds. However, when the three major shorebirds lineages (plovers and allies, sandpipers and allies, gulls and allies) were tested separately, a series of novel relationships were revealed. First, acrobatic aerial display flights, indicating male choice by females, are associated with increased species richness in the sandpipers and allies. Second, more polyandrous mating systems, suggesting increased sexual selection on females, are associated with increased species richness amongst the plovers and allies. Finally, increased fecundity is a marginal correlate of speciation in the gulls and allies, and notably, sexual selection and sexual conflict does not relate to speciation in this clade.

***(e) Population decline in North American shorebirds***

Shorebirds are declining worldwide – a matter of great concern since shorebirds are sensitive indicators of environmental change with their wide range of feeding and migration strategies (International Wader Study Group 2003). Habitat loss is likely to be a major contributor to the current crisis. Habitat loss, however, may not be the only factor since shorebirds may have different sensitivity to environmental changes. Of 200 populations with known trends, 48% are declining whilst the remainder are either stable (36%) or increasing (16%). Why are some species more prone to decline than others? Previous studies across birds suggest that long distance migration, small population and range sizes, slow life histories, and intense sexual selection are associated with greater extinction risk or more severe population declines (see Owens & Bennett 2000)

Why are some species more predisposed to population declines than others? In Chapter 7, I focus on recent declines in North American breeding shorebirds, and test how breeding systems, life-histories, migration, and ecology influence population trends. The major result was that species that migrate across continental North America are more prone to decline than species that do not. This is likely to be due to habitat change at key migratory stopover sites. Furthermore, more intense sexual selection, as indicated by large testis size, is associated with more severe population decline. The generality and broader significance of these results requires further investigation. Are all North American birds with cross-continental migratory routes more prone to decline? Do the



same patterns apply to shorebirds on a global scale? What are the relationships between evolutionary predisposition to decline and human effects?

## 5. CONCLUSIONS

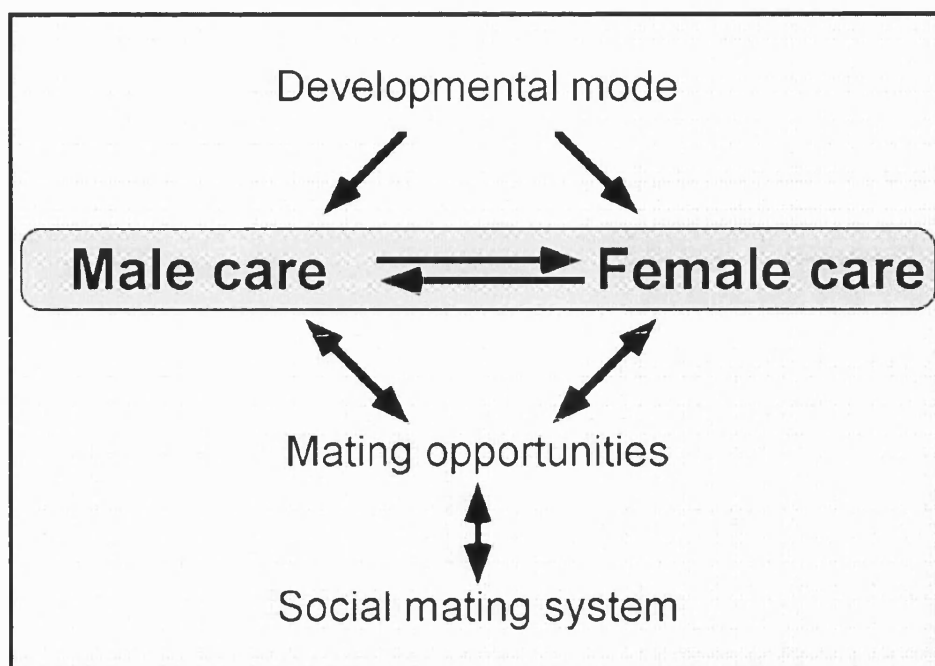
The broad aims of this thesis were to investigate the influence of sexual conflict on breeding systems and macroevolutionary patterns. To achieve this, I used shorebirds as model organisms and employed a range of phylogenetic comparative methods. I had two major objectives: first, to resolve the phylogenetic relationships between shorebird taxa and to construct a complete phylogeny of all extant shorebirds; and second, to use this phylogenetic framework to test a series of hypotheses relating sexual conflict to breeding system evolution, speciation, and extinction. Below, I discuss the extent to which these targets have been achieved.

Shorebird phylogeny has been the subject of sporadic discussion ever since the first major study by Strauch (1978), and the resolution of the interrelationships amongst plovers, sandpipers, gulls, and alcids had reached something of an impasse. This deadlock has been broken by the cytochrome-*b* and supertree studies presented here, in addition to two other studies of the nuclear genome (Ericson *et al.* 2003; Paton *et al.* 2003). Phylogenetic analyses of the cytochrome-*b* gene clearly show that the gulls and alcids are a monophyletic clade nested within the broad sandpiper lineage and this topology is supported by the supertree (see Chapters 2 & 3). Although some parts of the phylogeny remain poorly resolved, the nested position of the gulls and alcids has important implications for the way in which we approach cross-species studies of shorebirds.

Many cross-species studies of shorebirds exclude gulls and alcids (e.g. Whitfield & Tomkovich 1996; Reynolds & Székely 1997; Figuerola 1999). Gulls and alcids are often considered as being at the periphery of interest for shorebird enthusiasts. I argue that this can exclude potentially informative data and mask important patterns. For example, gulls and alcids may be considered paragons of moral virtue with almost exclusive biparental care and social monogamy, and as such uninformative in understanding the evolution of breeding systems. However, my analyses of

developmental mode in relation to parental care and social mating systems demonstrate that this is not the case. The predominance of social monogamy in gulls and alcids is likely the result of the semiprecocial developmental mode of their chicks. Thus, gulls and alcids show obligate biparental care and social monogamy. In contrast, the relaxation of parental constraint in precocial species indicates that monogamy is likely to be facultative. Such effects would likely be missed without due attention to all clades within the phylogeny. There may be cases where nested monophyletic clades can be excluded without detriment from comparative analyses, but this should not be standard practice and in general should be explicitly justified.

My results strongly suggest that sexual conflict has a major influence on both breeding system evolution and macroevolution. First, I have demonstrated that chick development influences the rate of phenotypic evolution of parental care and social mating system (as well as several life-history and ecological traits). Second, I have shown that parental care and social mating system have evolved in response to one another. Taken together, these results suggest that the ancient diversification of developmental mode determines whether desertion by either parent is possible (see fig. 4). Thus, in precocial species, potentially intense sexual conflict over parental care is expected. The decisions of each animal influence the mating opportunities available to the rest of the population, and thus determine the social mating system. However, the social mating system also influences mating opportunities, and consequently the optimal care decisions for the parents. These results are therefore highly consistent with a sexual conflict driven feedback loop determining the patterns of parental care and social mating system amongst shorebirds. It could be argued that shorebird breeding systems have evolved in a hierarchical fashion similar to that proposed by Bennett & Owens (2002), although this suggestion requires further investigation to assess the phylogenetic level at which parental care and social mating system is most variable amongst shorebirds.



**Figure 4.** A diagrammatic representation of the evolution of shorebird breeding systems by sexual conflict over care. Developmental mode constrains the potential for brood desertion. When this constraint is relaxed (i.e. in precocial taxa), social interactions over care and mating form a complex feedback loop that ultimately determines the social mating system of a species.

Sexual conflict over care also influences macroevolutionary patterns in shorebirds. First, sexual conflict was associated with species richness (conflict over mating optima; Chapter 6). It is notable that the factors influencing species richness are different in different lineages. Where the potential for diversification of parental care is high (i.e. in precocial taxa; see Chapter 3), species richness is associated with sexual conflict and differences in mating optima between the sexes. In contrast, where there is low potential for diversification of parental care (i.e. in semiprecocial taxa), fecundity, rather than sexual conflict, was the only correlate of species richness identified. Second, to a lesser extent, sexual conflict correlates with population decline (sperm competition; Chapter 7). The weaker association with population trend is likely due to a range of factors, involving both evolutionary predisposition to decline and contemporary problems (e.g. habitat loss and degradation, persecution, climate change), determining which species are more prone to decline. This is well illustrated by the finding that continental migrants are at greater risk of decline; it is likely that this is the case only because of substantial habitat loss along continental routes.

## 6. FUTURE DIRECTIONS

Taken together, the research presented herein has provided new insights into the role of sexual conflict as a general evolutionary mechanism and has advanced our understanding of shorebird diversity. However, important hypotheses remain to be tested pertaining to shorebirds specifically, and to the broader applicability of my results to other taxonomic groups.

First, there are unresolved parts of shorebird phylogeny: some taxa, such as the crab plover (*Dromas ardeola*), have not been well studied. This is important both in terms of understanding shorebird phylogeny, but also in the robustness of comparative analyses. Phylogenetic uncertainty is often regarded as a major limitation in phylogenetic comparative studies but this can be overcome by using modern phylogenetic techniques such as Bayesian analyses (Huelsenbeck *et al.* 2000). However, Bayesian methods for supertree construction are not yet well developed. Thus, large-scale phylogenetic analyses using modern molecular techniques and sequence data are highly desirable.

Second, key aspects of shorebird diversity remain to be explained. I have identified developmental mode as a possible constraint on sexual conflict over parental care, however, we do not know why developmental mode is itself so conservative. The difference between semiprecocial and precocial offspring is considered to be essentially behavioural (Starck & Ricklefs 1998), and based on this it seems reasonable to expect a great deal more variation between species than actually occurs. Several questions arise: how labile is developmental mode; do other factors, such as the growth rate of the chicks, differ between semiprecocial and precocial taxa?

In addition, I have shown that developmental mode may constrain the expression of sexual conflict, but the trade-off between males and females over care requires further investigation. Why do males in monogamous species frequently provide care for longer than females (e.g. dunlin)? This suggests that there is not a direct one-to-one compensation by one sex if the other deserts. Furthermore, if the female deserts before the male, why does the frequency of polyandry remain so low? Previous studies linked the duration of parental care to migration distance (Myers 1981; Reynolds & Székely 1997), although these were based on a limited range of species and crude data. It

remains to be tested whether migratory behaviour is a cause or consequence of conflict over parental care.

Perhaps the most pressing question regarding shorebird diversity is that of current population declines. I have shown that amongst North American shorebirds, both testis size and migration route correlate with population trends. However, shorebirds globally are in decline (International Wader Study Group 2003). Can this also be explained by migration route? In addition to understanding those factors that predispose some species to decline over others, there is an urgent need to assess the impact of human populations on the conservation status of shorebirds.

Finally, research into the causes and consequences of sexual conflict over care is in its infancy, and many of the issues addressed and raised throughout this thesis have not been tested at other taxonomic levels. It is not known whether sexual conflict over parental care is found in other avian orders, or across birds in general. Intuition suggests that some taxa are likely to exhibit a trade-off over care (ratites and tinamous, passerines) across species whereas others may not (albatrosses). Are the causes and consequences of sexual conflict consistent amongst birds? Studies across a broad range of taxonomic groups are required to understand how conflict influences social interactions, population trends and demographics, mating system evolution, life-histories and species richness.

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**Chapter 2.** *Phylogeny of shorebirds, gulls, and alcids (Aves: Charadrii) from the cytochrome-b gene: parsimony, Bayesian inference, minimum evolution, and quartet puzzling*

Gavin H. Thomas, Matthew A. Wills, and Tamás Székely (2004)  
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# Phylogeny of shorebirds, gulls, and alcids (Aves: Charadrii) from the cytochrome-*b* gene: parsimony, Bayesian inference, minimum evolution, and quartet puzzling

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## Abstract

Charadrii (shorebirds, gulls, and alcids) have exceptional diversity in ecological, behavioral, and life-history traits. A phylogenetic framework is necessary to fully understand the relationships among these traits. Despite several attempts to resolve the phylogeny of the Charadrii, none have comprehensively utilized molecular sequence data. Complete and partial cytochrome-*b* gene sequences for 86 Charadrii and five Falconides species (as outgroup taxa) were obtained from GenBank and aligned. We analyzed the resulting matrices using parsimony, Bayesian inference, minimum evolution, and quartet puzzling methods. Posterior probabilities, decay indices, and bootstrapping provide strong support for four major lineages consisting of gulls, alcids, plovers, and sandpipers, respectively. The broad structure of the trees differ significantly from all previous hypotheses of Charadrii phylogeny in placing the plovers at the base of the tree below the sandpipers in a pectinate sequence towards a large clade of gulls and alcids. The parsimony, Bayesian, and minimum evolution models provide strong evidence for this phylogenetic hypothesis. This is further corroborated by non-tree based measures of support and conflict (Lento plots). The quartet puzzling trees are poorly resolved and inconclusive.

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## 1. Introduction

The Charadrii (shorebirds, gulls, and alcids) display exceptional diversity in their ecology, life-history, and breeding systems (Bennett and Owens, 2002; Ligon, 1999; Reynolds and Székely, 1997; Székely et al., 2000). Phylogenetic comparative studies have begun to reveal relationships between sexual selection, breeding systems, migration, parental care, breeding density, and sexual size dimorphism. Despite advances in our knowledge of the life-history evolution of the group, the phylogeny itself remains unresolved.

The earliest studies of Charadrii classification differed markedly from contemporary views. The close relationships of sandpipers, avocets, oystercatchers, and plovers were recognised by Linnaeus (1758) in his group the Grallae, but he also included storks, herons, and

rails in this group whilst excluding the gulls and alcids. The affinities of the alcids initially proved elusive, being regarded as relatives of the penguins until Huxley (1867) grouped them with the gulls based on palatal type. Gadow (1892) proposed a classification of the Order Charadriiformes that resembles closely current schemes including sandpipers, plovers, gulls, and alcids. Despite this apparent intuition, the bustards (Otididae) were later grouped with the plovers and sandpipers because of their superficial similarity to thick-knees (Burhinidae), whilst the gulls and alcids were still excluded (e.g., Sharpe, 1896). Subsequent classifications involved minor amendments to previous schemes with a significant contribution coming from a series of detailed anatomical papers by Lowe (e.g., 1915, 1922). The extensive taxonomic works on the Charadrii have yielded surprising insights into the evolutionary history of the group. For example, Jehl (1968) presented a “tentative phylogeny” based on color patterns of the downy young, which closely resembles the more recent DNA–DNA hybridization phylogeny of Sibley and Ahlquist

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(1990). However, despite this long history of investigation into the affinities of Charadrii (a fuller review, from Linnaeus, 1758 to Dittmann et al., 1989, is provided by Sibley and Ahlquist, 1990), most recent phylogenetic studies have drawn on just one of two lines of evidence. These yielded conflicting trees.

Strauch (1978), using morphological data, produced the largest Charadrii phylogeny to date (227 taxa) and proposed that the alcids (Alcinae) are the most basal group, with the sandpipers (Scolopacida) constituting the sister group of a gull/plover (Larinae/Charadrii) clade. A series of reappraisals of Strauch's dataset (e.g., Björklund, 1994; Chu, 1995; Dove, 2000) found consistent results (but see Mickevich and Parenti, 1980). In contrast, using DNA–DNA hybridization data, Sibley and Ahlquist (1990) suggested a sister group relationship between the gulls and the alcids. They resolved the plovers as a sister group to the gull/alcid clade with the sandpipers falling at the base of the Charadrii.

More recently, Christidis et al. (1996), Joseph et al. (1999), and Whittingham et al. (2000) used DNA sequence data to infer the interrelationships of the sandpipers, the genus *Charadrius*, and the jacanas, respectively. However, DNA sequence data have not previously been used to infer the phylogeny of the suborder as a whole. Mitochondrial DNA, specifically the cytochrome-*b* gene has proven particularly useful in delineating genus and species level splits in previous phylogenetic studies of birds (e.g., the cranes, Gruidae; Krajewski and Fetzner, 1994). It is also the most widely sequenced gene available for the Charadrii from GenBank (Benson et al., 1998).

Here, we have collated cytochrome-*b* sequences from 86 Charadrii taxa, plus five outgroup taxa, to produce a dataset representing the whole group. We analyzed this dataset using four inference methods; namely, parsimony, Bayesian inference, minimum evolution (general time reversible model), and quartet puzzling (maximum likelihood). We provide measures of support for these hypotheses (bootstrapping and decay indices) as well as presenting Lento plots (a non-tree based approach; Lento et al., 1995) of the support and conflict of taxon splits to assess the validity of our hypotheses in relation to previous views of Charadrii phylogeny (as in Mallatt and Winchell, 2002).

## 2. Materials and methods

### 2.1. Taxa used and sequence alignment

DNA sequences from the cytochrome-*b* gene of 86 Charadrii species and five species of the Falconides (taxonomy based on Monroe and Sibley, 1993) were obtained from GenBank (Benson et al., 1998). Sequence length ranged from 258 bp to complete sequences of

1143 bp. We distinguish between two groups of taxa within our dataset. The first contains 41 taxa with sequences from 1020 to 1143 bp and the second contains 45 taxa with sequences from 258 to 345 bp. There are no taxa with sequence length in the range 346–1019 bp.

Missing character data are a significant problem in phylogenetic analysis (Wilkinson, 1995). Despite the reduced taxonomic coverage, we therefore use the dataset with longer sequence lengths (hereby referred to as the primary dataset) in all of our main analyses (following Rosenberg and Kumar (2001)). The taxa in the second group (hereby referred to as the expanded dataset; all of these taxa are missing at least 69% of the total possible characters) are included to further test the validity of our results and investigate the effects of taxon sampling. Using a taxonomically smaller dataset allowed for the calculation of decay indices for the strict consensus parsimony tree using *TreeRot* (Sorenson, 1999) and the total support index (TSI, Bremer, 1994).

All outgroup sequences (Falconides) were complete. We chose members of the Falconides as outgroup taxa because of their close relation to the Charadrii (Monroe and Sibley, 1993; Sibley and Ahlquist, 1990). A closely related outgroup is essential for avoiding the potential pitfall of long-branch attraction (Felsenstein, 1978; Hendy and Penny, 1989). A full list of taxa, GenBank accession numbers, and sequence lengths are provided in Appendix A.

We collated the 91 nucleotide sequences in FASTA format in the same open reading frame. These were translated to protein sequences and the program *PileUp* (Wisconsin Package, 2001) was used to align them. Minor adjustments to the alignment were made by eye. We used the program *AA2nuc* (courtesy of Prof. Laurence Hurst, University of Bath) to overlay the nucleotide sequences back onto the aligned proteins for subsequent analysis.

The 91 taxon data matrix in NEXUS format (Maddison et al., 1997) is available from EMBL (<http://www.ebi.ac.uk/embl/>) under the Accession No. ALIGN\_000529.

### 2.2. Phylogenetic analysis

We performed Bayesian analysis with the program *MrBayes* (Huelsenbeck and Ronquist, 2001) using the General Time Reversible (GTR) model of substitution with rate of heterogeneity set to a gamma distribution, hence applying the fewest possible number of constraints to the dataset. *Falco peregrinus* was used as the designated outgroup. The analysis was run with four chains for  $10^7$  generations and the current tree was saved to file every 100 generations. This generated an output of  $10^5$  trees. The  $-\log_e(\text{likelihood})$  stabilized after approximately  $10^5$  generations and the first  $10^4$  trees (10% “burn-in” in Bayesian terms) were discarded

as a conservative measure to avoid the possibility of including random, sub-optimal trees. We then used *PAUP\*4.0b10* (Swofford, 2000) to obtain a 50% majority-rule consensus tree. The percentage of samples recovering any particular clade in a Bayesian analysis represents that clade's posterior probability (Huelsenbeck and Ronquist, 2001). We follow the protocol of Leache and Reeder (2002) in considering support for probabilities of 0.95 or greater to be significant.

We carried out the maximum likelihood analysis in *Tree-puzzle* (Strimmer and von Haeseler, 1996) using a quartet puzzling algorithm to generate trees. The analysis was run with an unconstrained Tamura and Nei (1993) model of substitution with eight categories of gamma-distributed rates estimated from the dataset. *F. peregrinus* was set as the designated outgroup and we ran the analysis for  $5 \times 10^4$  puzzling steps. The transition/transversion ratio of 4.29:1 was estimated from the dataset.

Parsimony analysis was implemented in *PAUP\*4.0b10* (Swofford, 2000). Tree searches were heuristic, using tree-bisection-and-reconnection (TBR) branch swapping for 10 random addition sequence replicates. The transition/transversion weight estimate from the *Tree-puzzle* output file was incorporated by means of a step matrix. The number of parsimony informative characters in the dataset was 118 from first and second codon positions and 339 from third codon positions (a ratio of 1:2.87). Accounting for this, we took a conservative approach and assigned first and second codon positions a weighting of two, and third codon positions a weighting of one. Time constraints due to the large size of the expanded dataset precluded the calculation of support measures (e.g., decay indices, Bremer, 1988).

We constructed minimum-evolution trees in *PAUP\*4.0b10* (Swofford, 2000) using the same GTR model as in the Bayesian analysis. LogDet/paralinear distances (Lockhart et al., 1994) that do not assume stationary base frequencies were not used on our alignment of the primary dataset as we did not find evidence for non-stationary base frequencies ( $\chi^2_{135} = 152.409$ ,  $p = 0.145$ ).

Measures of tree support are limited by the size of the dataset (see Results) but we present bootstrap values for the Bayesian and minimum-evolution trees, and decay indices for the primary dataset parsimony tree. In addition, we used the program *Spectronet* (Huber et al., 2002; available from <http://awcmee.massey.ac.nz/spectronet/>) to estimate support and conflict within gene sequences for groups of taxa (referred to as splits). Support and conflict can be explained by considering an example of three sequences across five taxa (taken from Lento et al., 1995): sequence 1 = [G, G, A, A, A]; sequence 2 = [C, C, A, A, A]; sequence 3 = [C, T, C, T, T]. In sequence 1, taxa 1 and 2 share a guanine, whilst taxa 3–5 share an adenine. Sequence 1 supports a split of taxa 1 and 2 from taxa 3, 4, and 5. Sequence 2 supports the same split with taxa 1 and

2 sharing a cytosine, and taxa 3, 4, and 5 again sharing an adenine. Conflict occurs where a sequence is incompatible with the split in question. Sequence 3 is incompatible with sequences 1 and 2 because a split of taxa 1 and 3 (grouped together by sharing a cytosine) and taxa 2, 4, and 5 (grouped together by sharing a thymine) is evident. Support and conflict for all possible splits of data can be found in this way. Splits were determined directly by resolving nucleotides to either a purine or pyrimidine. We display the results as Lento plots (Lento et al., 1995). Only those splits with support values of two or more are presented here. It is important to note that each individual split can be incompatible with many others hence the frequency of conflict tends to be much larger than the frequency of support (Lento et al., 1995).

### 3. Results

#### 3.1. Bayesian inference

Fig. 1A shows the  $9 \times 10^4$  trees remaining after burn-in combined as a 50% majority-rule consensus tree. Figures on the resolved branches are posterior probabilities. The mean  $-\log_e(\text{likelihood})$  of these trees was  $-12586.48$ . In total, 31 (out of 39) nodes were significantly supported by their posterior probabilities, including several major clades (see Section 4). Resolution is high throughout the tree and ingroup monophyly is supported.

#### 3.2. Parsimony

Parsimony analysis slows down considerably as the number of taxa in the dataset is increased. To maximize the efficiency of our tree search, we produced a UPGMA tree to highlight taxa with identical or equivalent codings (that is, sister taxa with zero branch length) for safe taxonomic reduction (Wilkinson, 1995). Those taxa that do not have unique character codings may be safely deleted if an index taxon (an arbitrary choice if the codings are identical, but the most informative taxon if they are equivalent) is retained. Only one taxon, *Catharacta maccormicki*, was identified for deletion and its equivalent *Catharacta antarctica* was retained. Subsequent analysis produced two most parsimonious trees (MPTs) with a length of 5294.39 (consistency index, 0.3379; retention index, 0.0.6177; TSI, 0.1935). Ingroup monophyly was supported and resolution was generally high with the exception of some of the interrelationships of the sandpipers and gulls. The strict consensus tree, with decay indices, is shown in Fig. 1B.

#### 3.3. Maximum likelihood

The resolution of the quartet puzzling tree (not shown) was extremely poor and gives little meaningful

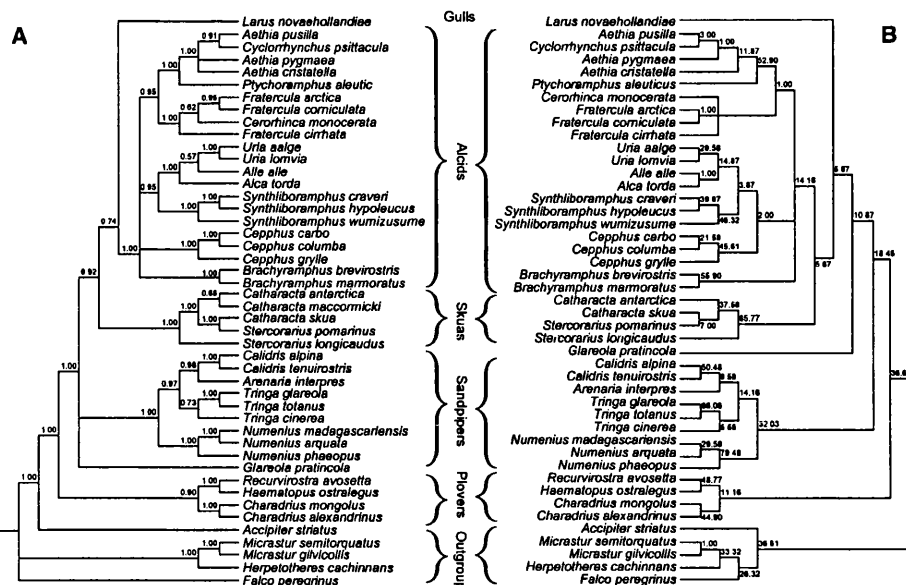


Fig. 1. Phylogenetic relationships of the Charadrii based on analysis of the primary cytochrome-b dataset using Bayesian (A) and parsimony (B) analyses. Posterior probabilities (A) and decay indices (B) for all resolved nodes are shown on internal branches. See text for further details.

information about the relationships of the Charadrii. This low resolution neither confirms nor refutes the monophyletic status of the ingroup.

### 3.4. Minimum evolution

Fig. 2A shows the minimum-evolution bootstrap tree (100 bootstrap replicates). Fig. 2B shows the minimum-evolution tree with branch lengths. Bootstrap values

indicate only weak support for many of the deep nodes, although support for ingroup monophyly is relatively high (88%).

### 3.5. Expanded dataset analysis

Using the expanded dataset did not alter the gross branching patterns (the respective groupings for sandpipers, plovers, gulls, and alcids) of the various trees.

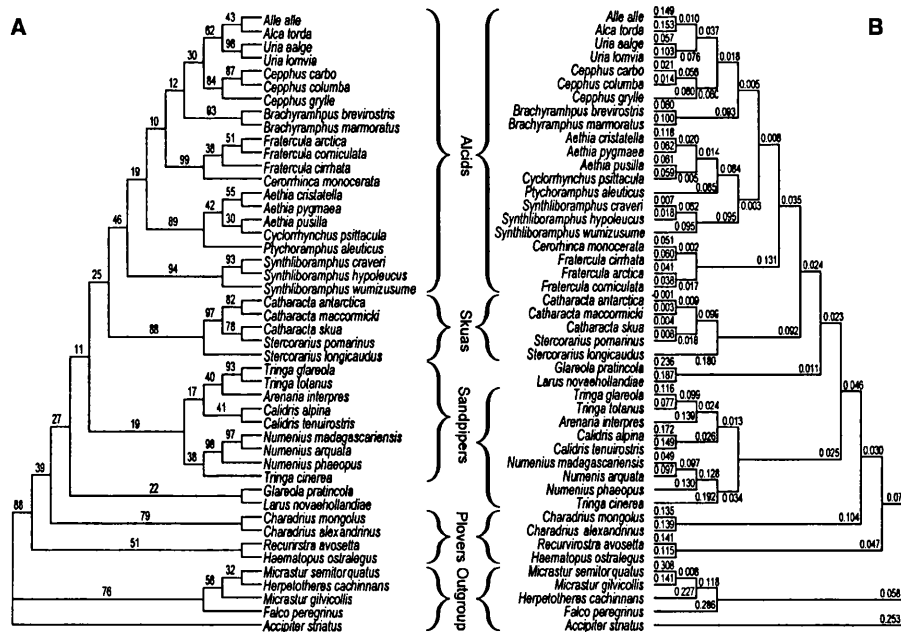


Fig. 2. Phylogenetic relationships of the Charadrii based on analysis of the primary cytochrome-b dataset using the minimum-evolution criterion under the GTR model. Bootstrap values (A) and branch lengths (B) on internal branches. See text for further details.



Fig. 3 shows the results from the expanded Bayesian analysis, revealing the same major branching patterns as the primary dataset, and bootstrap values are consistently high (34/62 with values  $\geq 0.95$ ). The addition of several gull taxa does not alter the monophyletic status of the clade, although resolution is poor. Similarly, an expanded parsimony analysis (Fig. 4) produced reduced resolution within the major clades (particularly amongst the sandpipers), but consistent relationships between them. Note that for the expanded parsimony analysis we tested for safe taxonomic deletion following the same approach taken for the primary

dataset. Six taxa were identified for deletion and their equivalents (in parentheses) were retained: *Larus ridibundus*, *Larus cirrocephalus*, (*Larus scopulinus*); *Larus occidentalis* (*Larus livens*); *Larus hyperboreus*, *Larus californicus*, (*Larus thayeri*); *C. maccormicki* (*C. antarcticus*). Bootstrap and decay indices could not be calculated for the parsimony tree because the expanded dataset is too large. The expanded minimum-evolution tree is not shown here because many pairwise distances could not be calculated due to missing data in the extra sequences and this resulted in a highly dubious tree. Resolution of the quartet puzzling tree was further

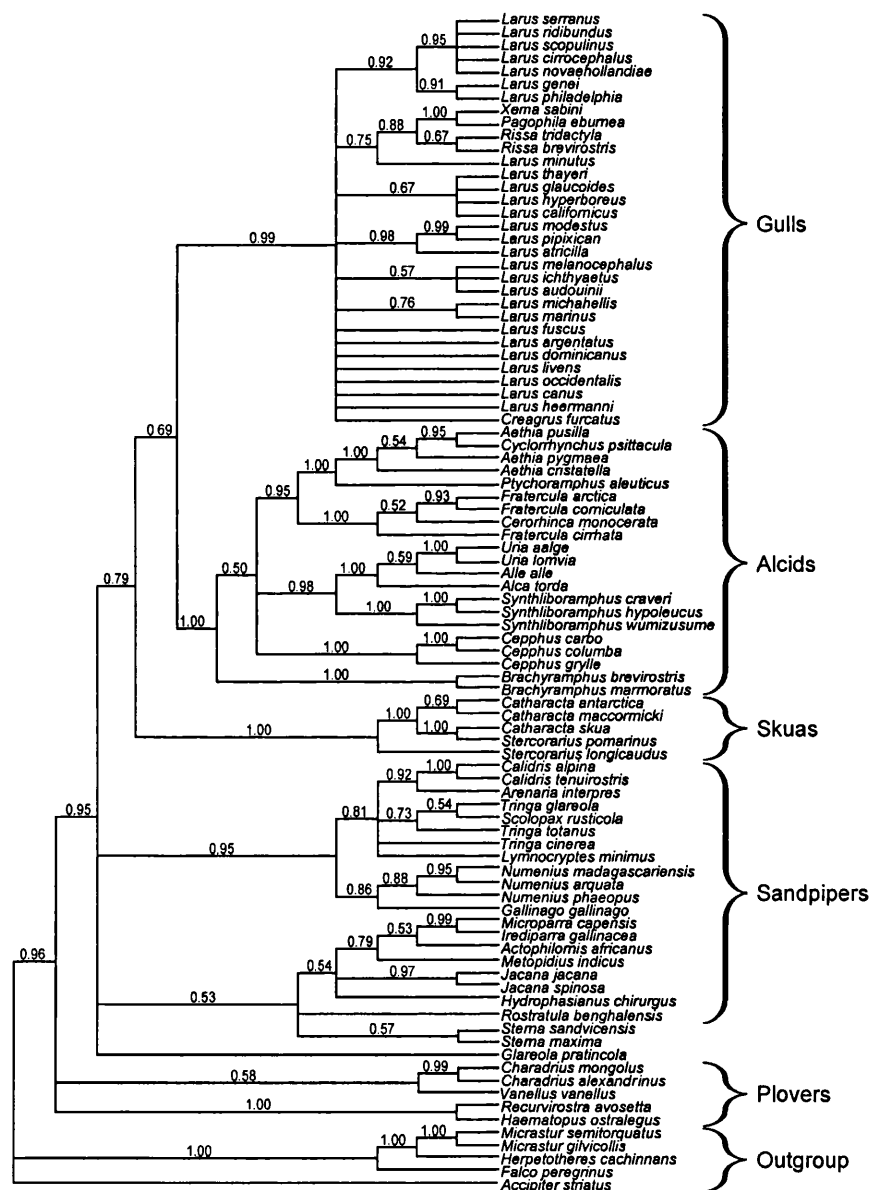


Fig. 3. Phylogenetic relationships of the Charadrii based on analysis of the expanded cytochrome-*b* dataset using Bayesian analysis. Posterior probabilities for all resolved nodes are shown on internal branches. See text for further details.

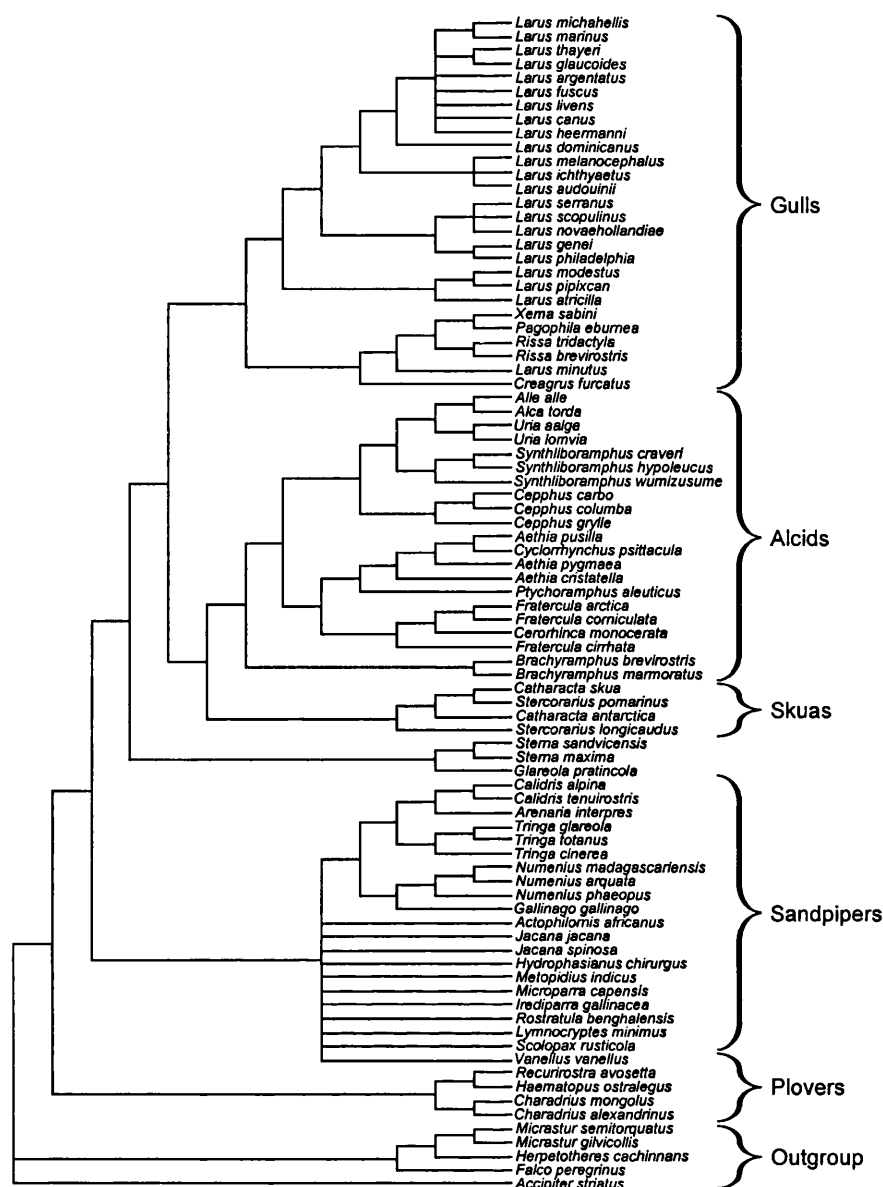


Fig. 4. Phylogenetic relationships of the Charadrii based on analysis of the expanded cytochrome-*b* dataset using parsimony analysis. See text for further details.

reduced and is again of little use in delineating the relationships within the Charadrii.

### 3.6. Support and conflict

Fig. 5 shows a Lento plot of support and conflict of the 16 most supported splits in the dataset. Single taxon splits are not shown. Support (bars above the x-axis) and conflict (bars below the x-axis) are given as a frequency of the number of supporting or conflicting sequences. Of particular interest is the support given to the monophyly of sandpipers (column I) and the grouping of sandpipers and plovers together (column O).

## 4. Discussion

### 4.1. Bayesian inference, parsimony, quartet puzzling, and minimum evolution analyses of Charadrii phylogeny

The use of Bayesian inference, parsimony, and minimum-evolution methods on the cytochrome-*b* gene of the Charadrii (using both the primary and expanded datasets) produced well-resolved trees supporting four distinct lineages consisting of gulls, alcids, plovers, and sandpipers. With the exception of the bootstrap minimum-evolution tree, the gulls are a sister group to the alcids. The alternative derived from the bootstrap minimum-evolution tree is a monophyletic group of

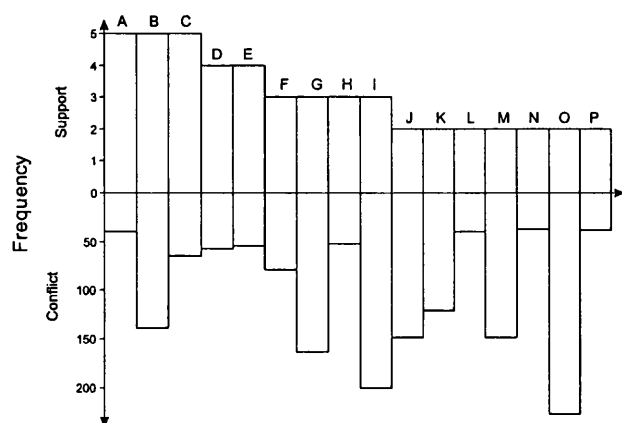


Fig. 5. Lento plot showing support and conflict frequencies for the 16 best supported splits in the primary cytochrome-*b* dataset. The taxa included in the splits are: (A) *Tringa glareola*, *Tringa totanus*; (B) *Haematopus ostralegus*, *Charadrius alexandrinus*, *Charadrius mongolus*, *Recurvirostra avosetta*; (C) *Numenius phaeopus*, *Numenius madagascariensis*, *Numenius arquata*; (D) *Calidris alpina*, *Calidris tenuirostris*; (E) *Haematopus ostralegus*, *Recurvirostra avosetta*; (F) *Aethia cristatella*, *Aethia pusilla*, *Aethia pygmaea*, *Cyclorhynchus psittacula*, *Ptychoramphus aleuticus*; (G) *Calidris alpina*, *Calidris tenuirostris*, *Tringa glareola*, *Tringa totanus*, *Tringa cinerea*, *Arenaria interpres*; (H) *Charadrius alexandrinus*, *Charadrius mongolus*; (I) *Calidris alpina*, *Calidris tenuirostris*, *Tringa glareola*, *Tringa totanus*, *Tringa cinerea*, *Arenaria interpres*, *Numenius phaeopus*, *Numenius madagascariensis*, *Numenius arquata*; (J) *Charadrius alexandrinus*, *Charadrius mongolus*, *Glareola pratincola*; (K) *Glareola pratincola*, *Tringa cinerea*; (L) *Uria aalge*, *Uria lomvia*; (M) *Charadrius alexandrinus*, *Charadrius mongolus*, *Tringa totanus*; (N) *Cephus carbo*, *Cephus grylle*, *Cephus columba*; (O) *Calidris alpina*, *Calidris tenuirostris*, *Tringa glareola*, *Tringa totanus*, *Tringa cinerea*, *Arenaria interpres*, *Numenius phaeopus*, *Numenius madagascariensis*, *Numenius arquata*, *Glareola pratincola*, *Haematopus ostralegus*, *Charadrius alexandrinus*, *Charadrius mongolus*, *Recurvirostra avosetta*; (P) *Synthliboramphus craveri*, *Synthliboramphus hypoleucus*, *Synthliboramphus wumizusume*.

alcids and sandpipers; however, support for this clade is very low (11%). Furthermore, analyses of support/conflict provide no corroborative evidence for such a split.

Bayesian and parsimony methods are highly consistent with regard to the relationships within the plovers and sandpipers, these splits are also well supported by analysis of support/conflict (columns I and O, Fig. 5). Similarly, Bayesian and parsimony methods are consistent in the interrelationships of the alcids. The only area of conflict is the position of *Alca torda* as either at the base of a clade consisting of *Alle alle*, *Uria lomvia*, and *Uria aalge* (Bayesian tree, Fig. 1A) or as the sister species of *A. alle* in the same four species clade (parsimony tree, Fig. 1B). Minimum evolution is consistent with parsimony analysis in its resolution of this clade.

The only part of the tree showing substantial conflict across methods is the clade of skuas. It is unclear whether they are basal to the alcids and gulls as in the Bayesian tree, or whether the gulls are in fact basal to an alcid/skuas clade (parsimony and minimum evolution).

Support/conflict analyses (Fig. 5) provided no further evidence for either hypothesis.

Expanding the dataset to include incomplete cytochrome-*b* sequences had little major impact on the broad structure of the phylogeny but some problems with using shorter sequences were revealed. The four main clades displayed in the expanded Bayesian and parsimony analyses reveal that only the alcids are consistently monophyletic. The position of two members of the gull clade (the terns, *Sterna sandvicensis* and *Sterna maxima*) is uncertain, but both sequences are incomplete (only 291 and 290 bp, respectively). Bayesian inference reports them within an unresolved polytomy consisting of the Greater Painted-snipe (*Rostratula benghalensis*) and several members of the Jacanidae, whereas the parsimony analysis places these terns with the Collared Pratincole (*Glareola pratincola*) at the base of a large clade consisting of all other gulls and alcids.

As in the primary dataset, the expanded parsimony analysis resolves the skuas at the base of the alcids with an exceptionally high decay index of 65.77, whereas the Bayesian analysis infers a position at the base of the collective gull/alcid clade with a significant posterior probability of 1.00. The respective support values for the clade mean that the position of the skuas cannot be conclusively determined from this analysis. Additional differences in topology between the Bayesian and parsimony results occur within the gull/alcid clade. The gulls in particular are very poorly resolved in the Bayesian analysis but parsimony analysis elucidates this group more fully. However, as these differences are attributable to variation in resolution, we conclude that there are no major areas of direct conflict between methods.

Variation in resolution also applies to the plovers and sandpipers but again there are few directly conflicting signals. The well-supported sister group relationships (e.g., *Calidris alpina*, *Calidris tenuirostris*; *Charadrius mongolus*, *Charadrius alexandrinus*) occur in both the Bayesian and parsimony analyses. However, in the parsimony tree, the Northern Lapwing (*Vanellus vanellus*) falls in an unresolved polytomy within the broad sandpiper clade, compared to the Bayesian analysis where it is resolved as a sister group to the two *Charadrius* species. Bayesian support is low (posterior probability of 0.58) for the three-taxon clade and we attribute its uncertain position to missing data (sequence length of only 300 bp).

In contrast to the alternative methods, quartet puzzling resulted in trees of strikingly poor resolution regardless of the size of dataset used, with the alcids forming the only distinct group (the internal relationships of which are unresolved). Atchley et al. (2001) also reported conservative (i.e., poorly resolved) results based on quartet puzzling. Strimmer and von Haeseler (1996) use the term *bad quartet* to describe cases where

there is no single quartet with a significantly better maximum likelihood value than the other possible outcomes, leading to poorly resolved trees. They show that a dataset with as few as 10–15% bad quartets is enough to result in incomplete resolution. Ranwez and Gascuel (2001) also cautioned that quartet methods are particularly sensitive to 4-tree inference error (that is, errors occurring in the production of the maximum likelihood four taxon quartet trees). They cite a 12-taxon example with variation in the outcome of only nine quartets (2% of the 495 possible outcomes), and state that this is sufficient to infer a wrong tree. Our cytochrome-*b* data contained 9.6% and 18.2% bad quartets for the primary and expanded datasets, respectively; hence, it is reasonable to expect reduced resolution in this case. Whilst Strimmer and von Haeseler (1996) refer to unspecified “background noise” obscuring the phylogenetic signal, Ranwez and Gascuel (2001) specifically cite long-branch attraction for the inference of variable quartets and hence as a significant reason for poor resolution of the overall tree. Furthermore, phylogenies of four taxon trees are known to be difficult to estimate if rates of evolution are high (Hillis et al., 1994). Despite this being the case for the mitochondrial genome as a whole (Yang, 1998), the cytochrome-*b* gene itself evolves at a more conservative rate than most other mitochondrial genes (Russo et al., 1996). Hence, we conclude that missing data contributes to the poor resolution of Charadrii phylogeny from the complete cytochrome-*b* dataset when using quartet puzzling methods, but that long-branch attraction may also be a major contributory factor, as is shown by the continued lack of resolution in the reduced dataset. As a note of caution, we acknowledge that Willson (1999) suggested that long-branch attraction was a lesser problem with quartet puzzling than with parsimony reconstruction.

#### 4.2. Comparison with DNA–DNA hybridization and morphological data

Sibley and Ahlquist (1990) proposed a shorebird phylogeny with the gulls and alcids in a sister group relationship. The sandpipers are the most basal lineage with the plovers forming a sister group to the gull–alcids clade. Sibley and Ahlquist received much criticism for both the methodological limitations (e.g. Houde, 1987; Springer and Krajewski, 1989) and results (e.g., Harshman, 1994) of their extensive study. Nonetheless, it remains an important and valid hypothesis of Charadrii phylogeny with which to compare new evidence.

There are several differences between the set of taxa used by Sibley and Ahlquist (1990) and the set used in our study, most notably we have more alcids but fewer plovers. However, the same major clades (gulls, alcids, plovers, sandpipers) are evident in both. The tree topologies based on the cytochrome-*b* gene indicate sup-

port for Sibley and Ahlquist’s (1990) gull–alcid clade, but we note that the support for this clade in the Bayesian tree is not significant (posterior probabilities of 0.74 excluding skuas, 0.92 including skuas for the primary dataset, and 0.69 and 0.79, respectively, for the expanded dataset). In addition, the unstable position of *S. sandvicensis* and *S. maxima* in the expanded analyses precludes the monophyly of this group. In contrast, most morphological data suggest that alcids are basal to all Charadrii and that gulls are a sister group to the plovers (Chu, 1995; Dove, 2000; Strauch, 1978). However, Björklund’s (1994) placing of the plovers and sandpipers in a sister group is consistent with our results, although the overall topology differs significantly in that Björklund placed the gulls and alcids at the base of the tree.

Our dataset comprises 86 out of 365 Charadrii taxa based on Monroe and Sibley (1993). However, several authors have suggested that taxon sampling may not be a significant problem in phylogenetic inference. Poe (1998) demonstrated that the number of taxa sampled has no significant impact on tree length based on a study of 29 datasets. Moreover, Rosenberg and Kumar (2001) suggested that longer sequences rather than extensive taxon sampling would improve the accuracy of phylogenetic inference. We acknowledge that Poe’s (1998) analysis was based on datasets of 20 or fewer taxa; however, the topologies presented here show no major differences between the primary and expanded datasets.

Assessing which analysis produces the most accurate hypothesis of Charadrii phylogeny is not trivial. Typically, we might assume support for a phylogenetic hypothesis if alternative lines of evidence produce the same (or similar) results (Miyamoto and Fitch, 1995). For the Charadrii, we have three apparently distinct datasets (cytochrome-*b*, morphology, and DNA–DNA hybridization) producing markedly different topologies (but see Kluge, 1998 for a discussion of data partitions). A major deficiency of previous phylogenetic studies of the Charadrii is the absence of accurate measures of tree stability. Chu (1995) reports the consistency index (CI) to assess the level of homoplasy and the fit of the data to the tree, but this is dependent on the number of characters and taxa in a dataset and is not directly comparable between trees derived from different data (Siebert, 1991). Dove (2000) uses a second tree statistic, the retention index (RI, Farris, 1989), which is superior to the CI in that it measures homoplasy as a fraction of the maximum possible homoplasy (Siebert, 1991). However, neither statistic says anything about how robust the tree is. Here, we have presented three independent analyses (Bayesian inference, parsimony, and minimum evolution), each with their own alternative measures of support (posterior probabilities, decay indices, and bootstrapping) that provide corroborative evidence for the major splits in Charadrii phylogeny. A fourth

analysis (maximum likelihood with quartet puzzling) is not conflicting. Furthermore, we also present Lento plots as a non-tree based measure of support and conflict.

The consistency with which a gull/alcid clade is resolved from cytochrome-*b* from both tree based (Bayesian inference, parsimony, minimum evolution) and non-tree based (support/conflict analyses, displayed as a Lento plot) methods suggest that our results fit more closely to the hypothesis of Sibley and Ahlquist (1990) than to any other large scale study of Charadrii phylogeny. However, it is also evident that there is still considerable conflict in the placement of gulls and alcids within the Charadrii as a whole. The suitability of the cytochrome-*b* gene for resolving these deep nodes may be questioned because of the possibility of saturation at third codon positions and limited variation in first and second codon positions (Meyer, 1994). Although the cytochrome-*b* gene typically evolves more slowly than other mitochondrial genes (Russo et al., 1996) it may still be too rapid for resolving relationships beyond the generic or at best family level in the Charadrii. The consistency of the phylogeny based on the cytochrome-*b* gene with previous studies (e.g., DNA–DNA hybridization, Sibley and Ahlquist, 1990; osteology, Strauch, 1978) in supporting monophyletic clades of plovers, sandpipers, gulls, and alcids suggests that it is effective at delineating species and genus level splits in the Charadrii. However, the lack of strong support for the relationships of the four main groups to one another points to a deficiency in accurately reconstructing deeper nodes. It may therefore prove fruitful to sequence genes that are more conservative, such as small-subunit ribosomal RNA, to resolve the present conflict in the relative positions of the plovers, sandpipers, gulls, and alcids.

#### 4.3. Conclusions

We have demonstrated strong support for the major clades of Charadrii using three different tree reconstruction criteria, and can therefore be confident that our phylogeny is reliable for the cytochrome-*b* sequence data. However, several areas of the tree are unresolved (many of the interrelationships of the gulls in particular), and not all sequences used are complete. Furthermore, only 86 taxa (from a possible 365 according to Monroe and Sibley, 1993) are included in the expanded dataset, leading to questions over whether our taxon sample is sufficient to produce a reliable measure of phylogeny.

Our results may be supplemented and improved in several ways. Additional sequences of cytochrome-*b* for many taxa, particularly the under-represented plovers, are desirable. Sequencing different genes may also improve both the taxonomic range and resolution of the deeper relationships in Charadrii phylogeny (see Peck

and Baker, 2001). Furthermore, a total evidence analysis including our cytochrome-*b* data along with morphological characters may be beneficial in order to clarify further the evolutionary relationships of this enigmatic group. Nonetheless, this analysis demonstrates the value of using multiple methods for phylogenetic inference and provides interesting new hypotheses of Charadrii phylogeny.

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#### Appendix A. Cytochrome-*b* taxon list

##### A.1. Ingroup taxa

*Actophilornis africanus* (GenBank Accession No.: AF146616; sequence length, 345 bp), *Aethia cristatella* (U37087; 1045), *Aethia pusilla* (U37104; 1045), *Aethia pygmaea* (U37286; 1045), *Alca torda* (U37288; 1045), *Alle alle* (U37287; 1045), *Arenaria interpres* (AF417928; 1143), *Brachyramphus brevirostris* (U37289; 1045), *Brachyramphus marmoratus* (U37290; 1045), *Calidris alpina* (U34686; 1045), *Calidris tenuirostris* (AF417924; 1143), *Catharacta antarctica* (U76792; 1020), *Catharacta maccormicki* (U76799; 1020), *Catharacta skua* (U76807; 1020), *Cephus carbo* (U37292; 1045), *Cephus columba* (U37293; 1045), *Cephus grylle* (U37294; 1045), *Cerorhinca monocerata* (U37295; 1045), *Charadrius alexandrinus* (AF417931; 1143), *Charadrius mongolus* (AF417927; 1143), *Creagrus furcatus* (AF268519; 290), *Cyclorhynchus psittacula* (U37296; 1045), *Fratercula arctica* (U37297; 1045), *Fratercula cirrhata* (U37298; 1045), *Fratercula corniculata* (U37299; 1045), *Gallinago gallinago* (AF194446; 307), *Glareola pratincola* (U37300; 1045), *Haematopus ostralegus* (AF440782; 1143), *Hydrophasianus chirurgus* (AF146619; 345), *Irediparra gallinacea* (AF146622; 344), *Jacana jacana* (AF146617; 345), *Jacana spinosa* (AF146618; 345), *Larus argentatus* (AF268495; 274), *Larus atricilla* (AF268509; 306), *Larus audouinii* (AF268514; 277), *Larus californicus* (AF268503; 308), *Larus canus* (AF268504; 294), *Larus cirrocephalus* (AF268518; 294), *Larus dominicanus* (AF268497; 308), *Larus fuscus* (AF268494; 276), *Larus genei* (AF268513; 308), *Larus glaucooides* (AF268499; 308), *Larus heermanni* (AF268506; 290), *Larus hyper-*

*boreus* (AF268500; 308), *Larus ichthyaetus* (AF268511; 308), *Larus livens* (AF268501; 308), *Larus marinus* (AF268496; 277), *Larus melanocephalus* (AF268510; 289), *Larus michahellis* (AF268493; 258), *Larus minutus* (AF268524; 307), *Larus modestus* (AF268507; 301), *Larus novahollandiae* (U37301; 1045), *Larus occidentalis* (AF268502; 308), *Larus philadelphia* (AF268517; 300), *Larus pipixcan* (AF268508; 291), *Larus ridibundus* (AF268515; 279), *Larus scopulinus* (AF268516; 307), *Larus serranus* (AF268512; 297), *Larus thayeri* (AF268498; 308), *Lymincryptes minimus* (AF194447; 293), *Metopidius indicus* (AF146620; 345), *Microparra capensis* (AF146621; 344), *Numenius madagascariensis* (AF417925; 1143), *Numenius arquata* (AF417929; 1143), *Numenius phaeopus* (AF417930; 1143), *Pagophila eburnea* (AF268521; 307), *Ptychoramphus aleuticus* (U37302; 1045), *Recurvirostra avosetta* (AF417926; 1143), *Rissa brevirostris* (AF268523; 265), *Rissa tridactyla* (AF268522; 299), *Rostratula benghalensis* (AF146623; 345), *Scolopax rusticola* (AF172384; 300), *Stercorarius longicaudus* (U76820; 1020), *Stercorarius pomarinus* (U76814; 1020), *Sterna maxima* (AF268526; 290), *Sterna sandvicensis* (AF268525; 291), *Synthliboramphus craveri* (U37304; 1045), *Synthliboramphus hypoleucus* (U37305; 1045), *Synthliboramphus wumizusume* (U37306; 1045), *Tringa glareola* (AF417923; 1143), *Tringa totanus* (AF417932; 1143), *Uria aalge* (U37307; 1045), *Uria lomvia* (U37308; 1045), *Vanellus vanellus* (AF172383; 300), *Xema sabini* (AF268520; 290), *Tringa cinerea* (listed as *Xenus cinereus*, AF417922; 1143).

## A.2. Outgroup taxa

*Accipiter striatus* (U83305; 1143), *Falco peregrinus* (U83307; 1143), *Herpetotheres cachinnans* (U83319; 1143), *Micrastur gilvicolis* (U83315; 1143), *Micrastur semitorquatus* (U83314; 1143).

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### **Chapter 3.** *A supertree approach to shorebird phylogeny*

Gavin H. Thomas, Matthew A. Wills, and Tamás Székely (2004)

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Research article

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## A supertree approach to shorebird phylogeny

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### Abstract

**Background:** Order Charadriiformes (shorebirds) is an ideal model group in which to study a wide range of behavioural, ecological and macroevolutionary processes across species. However, comparative studies depend on phylogeny to control for the effects of shared evolutionary history. Although numerous hypotheses have been presented for subsets of the Charadriiformes none to date include all recognised species. Here we use the matrix representation with parsimony method to produce the first fully inclusive supertree of Charadriiformes. We also provide preliminary estimates of ages for all nodes in the tree.

**Results:** Three main lineages are revealed: i) the plovers and allies; ii) the gulls and allies; and iii) the sandpipers and allies. The relative position of these clades is unresolved in the strict consensus tree but a 50% majority-rule consensus tree indicates that the sandpiper clade is sister group to the gulls and allies whilst the plover group is placed at the base of the tree. The overall topology is highly consistent with recent molecular hypotheses of shorebird phylogeny.

**Conclusion:** The supertree hypothesis presented herein is (to our knowledge) the only complete phylogenetic hypothesis of all extant shorebirds. Despite concerns over the robustness of supertrees (see Discussion), we believe that it provides a valuable framework for testing numerous evolutionary hypotheses relating to the diversity of behaviour, ecology and life-history of the Charadriiformes.

### Background

The shorebirds and allies (Aves: Charadriiformes; [1]) present an exceptional group for studying numerous evolutionary hypotheses. Their remarkable diversity of social mating system, parental care, sexual dimorphism, ecology and life-history make them an ideal group for unravelling the mechanisms of, for example, sexual selection and sexual conflict. Previous comparative studies have made significant contributions to our understanding of the evolution of mating systems [2], parental care [3,4], sexual size dimorphism [5-7], locomotion and morphology [8], migratory behaviour [9], egg size [10], and plumage

colouration [11]. The importance of phylogeny in cross-species comparative studies is well documented [12-14]. Large and well-resolved phylogenies that incorporate divergence times provide powerful tests of a wide range of hypotheses whilst accounting for the effects of shared evolutionary history [13,15]. However, the shorebird studies listed above were limited by the lack of a complete phylogeny for the group. Most of these studies are based on derivations of the seminal work of Sibley and Ahlquist [16], yet this study included less than a quarter of extant and recently extinct shorebird species. Recently extinct taxa (according to Monroe and Sibley [1]) are: the

Tahitian sandpiper *Prosobonia leucoptera*, the Canary Islands oystercatcher *Haematopus maedewaldoi*, and the Great auk *Pinguinus impennis*.

Recent molecular studies covering a wide range of shorebird families have drawn attention to conflict in the reconstruction of the deep basal nodes of shorebird phylogeny (figure 1; reviewed by van Tuinen *et al.* [17]). For example, morphological data [18,19] places Alcinae (auks, puffins, murre) at the base of the shorebird tree whilst sequence [20-22] and DNA-DNA hybridisation [16] data suggests that they are a highly derived sister group to Stercorariini (skuas and jaegers), Larini (gulls), Sternini (terns), and Rynchopini (skimmers). It is important to note that taxon coverage differs between these studies and this may be an important factor in determining the tree topology. Specific phylogenies have been derived, for example, for sandpipers [23], the genus *Charadrius* [24], and jacanas [25] using DNA sequence data. In contrast, morphological evidence provided the basis for Chu's [26] study of gull phylogeny. Strauch [18] presented the most complete data set of 227 Charadriiformes species. However, despite the plethora of cladograms for particular shorebird groups (see reviews by Sibley and Ahlquist [16]; Thomas *et al.* [22]), those that address relationships across the whole clade use either sparse taxon sampling [16,27], or are based on reassessments of Strauch's [18] data [19,28-30]. Note that Dove [30] included a feather microstructural analysis in addition to her reanalysis of Strauch's [18] data.

#### Combining phylogenetic data

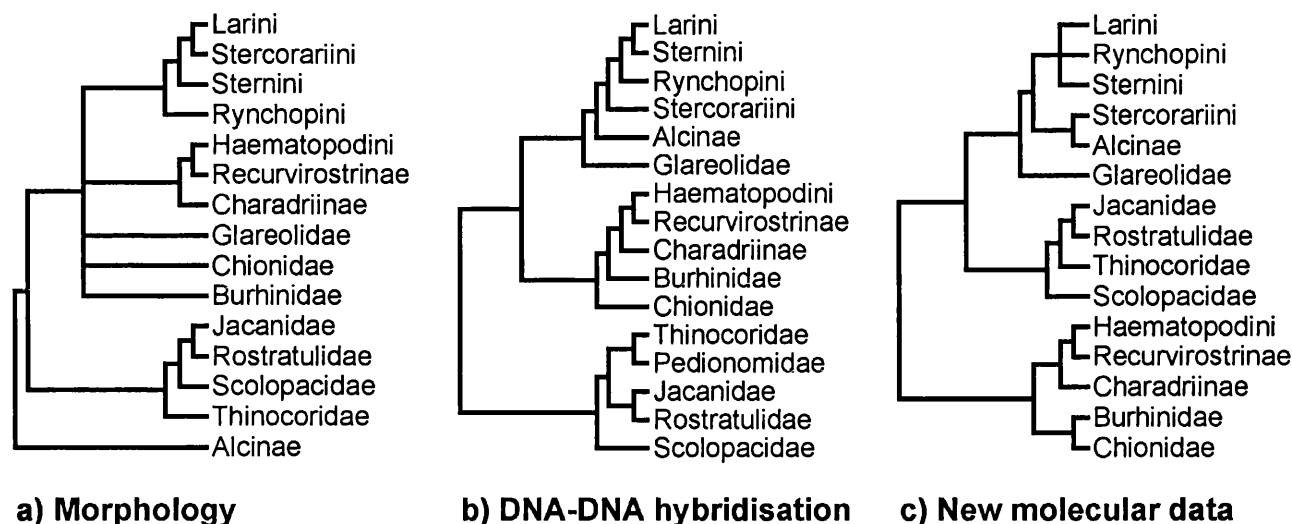
Numerous methods and types of data can be used to infer phylogeny. Frequently, as in Charadriiformes, a single analysis incorporating all taxa of interest is absent. Under the principle of total evidence [31], all sources of phylogenetic information should be combined to maximize their explanatory power. Eernisse and Kluge [32] define total evidence as a method for seeking the best fitting phylogenetic hypothesis for an unpartitioned set of synapomorphies (shared derived characters) using character congruence (characters combined in a supermatrix). Hence, this method combines the primary data (molecular, morphological and behavioural characters) into a single analysis. The approach is powerful because weak signals in the partitioned data sets may be enhanced when combined, and previously obscured relationships may be revealed [33].

The total evidence approach has both practical and theoretical problems. First, only certain types of data can be combined. For example, nucleotide sequences and morphological traits can be readily assessed together as characters, but it is not generally possible to include nucleotide sequences and genetic distance data in a single

analysis [34]. We acknowledge that Lapointe *et al.* [35] suggest a distance based approach to combine otherwise incompatible data in a total evidence analysis, although this method has not been tested beyond a single application. The consequence is that it is rarely possible to combine all sources of data in practice and the lack of overlap in combinable data sets may result in a reduction of the number of taxa included. Second, Miyamoto and Fitch [36] contend that combining data sets is rarely justified because partitions of phylogenetic data are real and unequivocal. They argue that several partitions producing similar topologies provide multiple lines of independent evidence supporting that topology.

Theoretical arguments over the benefits of total evidence will undoubtedly continue, but perhaps the major barriers to its use are the often very high computational demands of large matrices, and the *a priori* exclusion of certain data types. This is particularly true of Charadriiformes phylogeny, where one of the most significant contributions to the field – DNA-DNA hybridisation – cannot be included. An alternative set of techniques, collectively termed supertrees (e.g., Matrix Representation with Parsimony, MRP; [37,38]), enables combination of trees (rather than raw data) from otherwise incompatible sources. MRP methods code source phylogenies based on the presence and absence of taxa at each node of the tree [37-39] and are thus one step removed from the primary data. It is important to recognise that supertrees should not be regarded as a replacement for exhaustive phylogenetic studies of the primary data and there are drawbacks to the methods (see Discussion). However, they do enable very large phylogenies to be constructed rapidly [15]. Supertrees have been constructed successfully for a wide variety of taxa including carnivores [15], primates [39], seabirds [40], dinosaurs [41], and grasses [42].

Shorebirds are particularly well suited for supertree treatment, since there are numerous incomplete phylogenies available and a broader phylogeny is desirable to facilitate powerful analyses of numerous evolutionary hypotheses (see above). Here, we present the first complete composite phylogeny of extant and recently extinct [1] shorebirds using the MRP approach. We are therefore combining data on tree topologies, and not conducting a simultaneous analysis on the original data. We also use fossil and molecular data to estimate divergence times (see Methods). The combination of complete taxonomic coverage and the inclusion of branch lengths provide the basis for future comparative analyses of Charadriiformes evolution. In addition, conflicting and unresolved areas of Charadriiformes phylogeny are revealed.



**Figure 1**  
**Previous hypotheses shorebird phylogeny.** Family and subfamily level relationships of shorebirds based on: a) Morphological data [19]; b) DNA-DNA hybridisation [16]; c) Sequence analysis of RAG-I [20, 21], cytochrome-b [22] and myoglobin intron II [21].

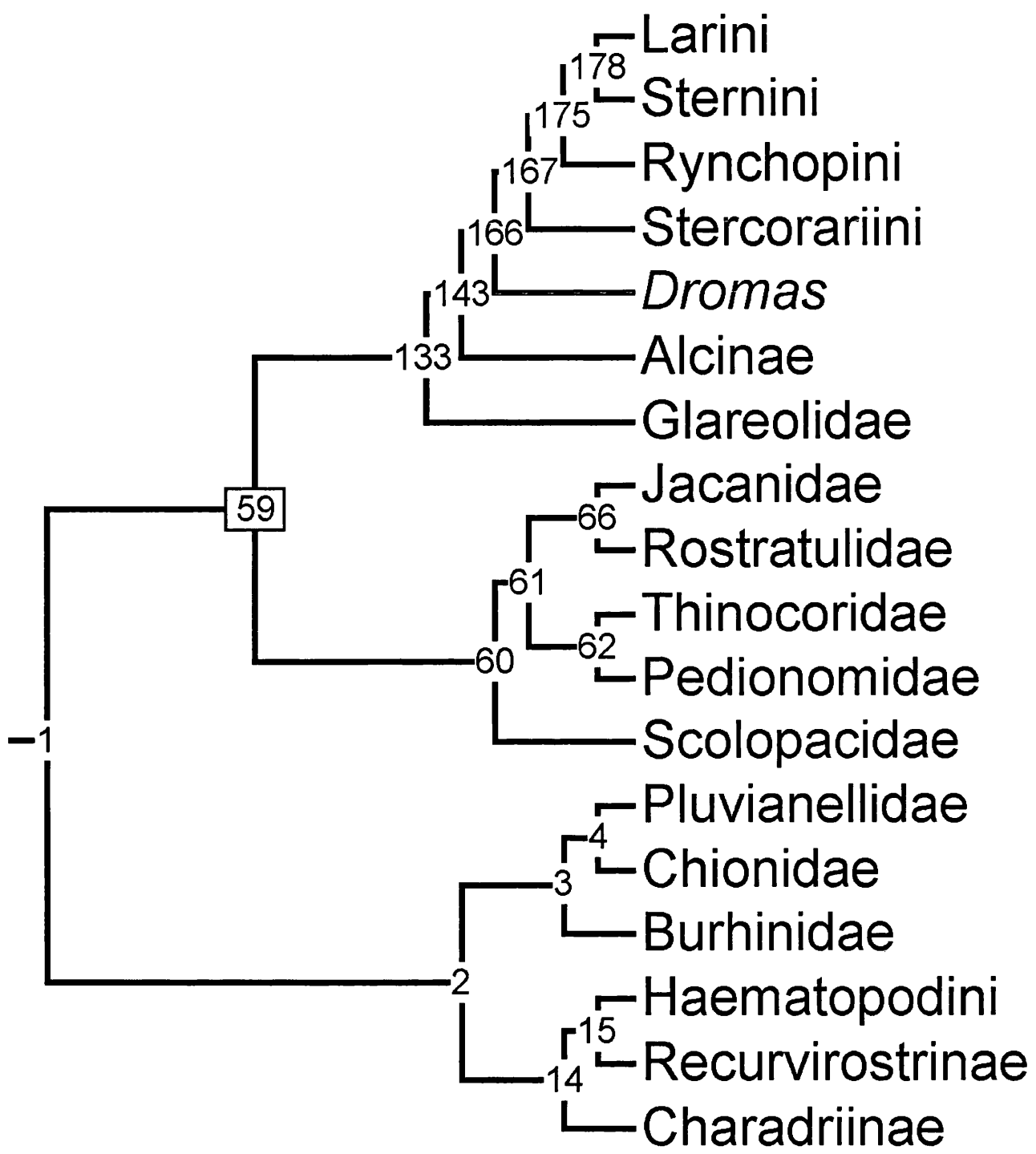
## Results and Discussion

### Supertree resolution and topology

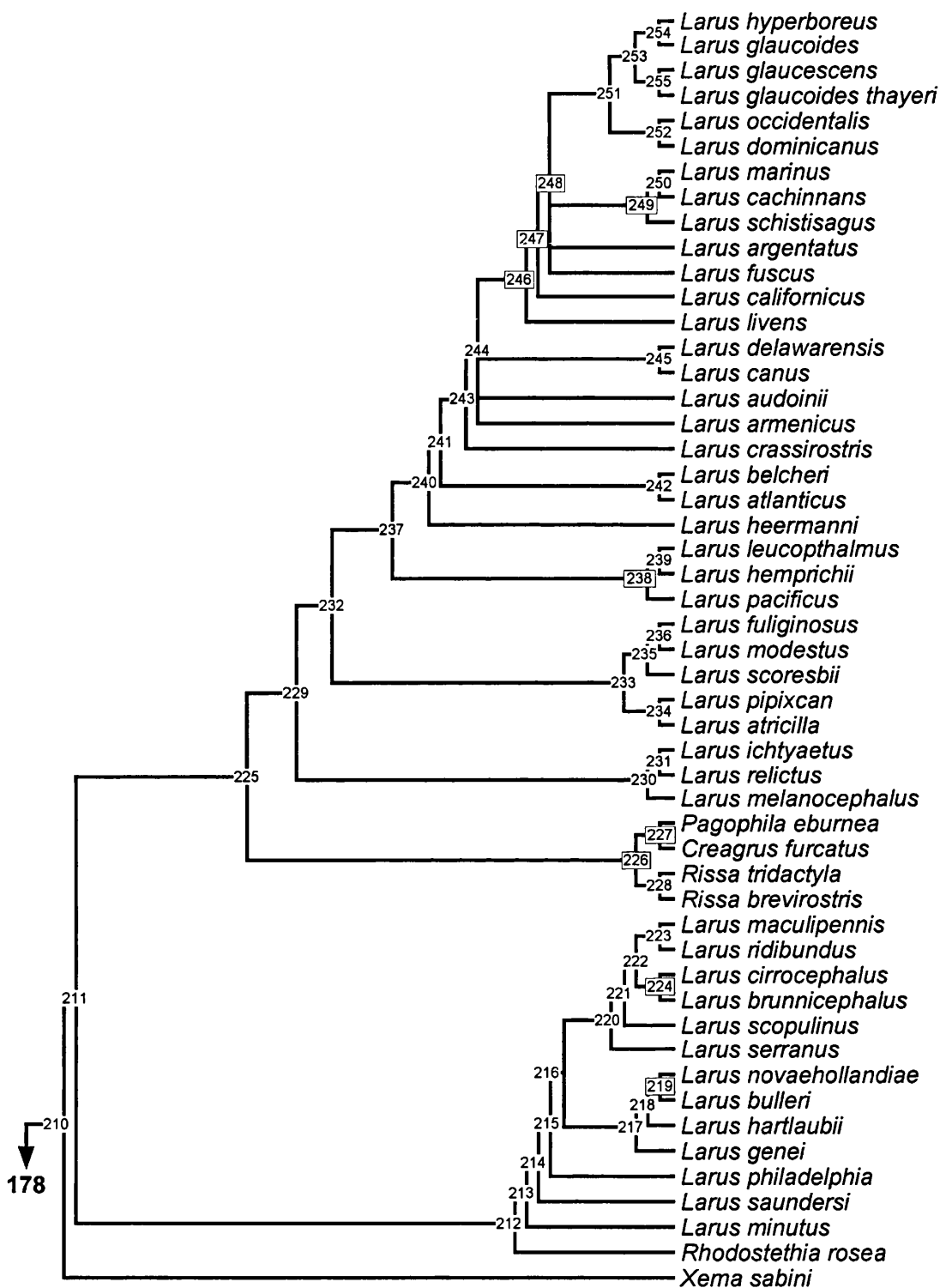
We found 1469 equally short trees of length 1847 steps using the parsimony ratchet approach (see Methods). This compares favourably to a standard heuristic search that yielded shortest trees of 1853 steps. All subsequent results and discussion refer to the parsimony ratchet analyses. Figure 2 shows the family and subfamily level relationships of shorebirds based on the strict and 50 % majority-rule consensus tree (see additional file 1 for branch length estimates). Figures 3,4,5,6,7,8,9 show the species level phylogeny. The full 50% majority rule consensus and the strict consensus trees are available as additional file 2 and 3 respectively. The 50% majority-rule consensus tree is well resolved (73.1%; 255 nodes out of a possible 349 in a fully bifurcating tree), although the strict consensus tree is only 49.6% resolved (173 from 349 possible nodes). The majority rule tree includes nine novel clades (numbers 20, 29, 57, 85, 89, 108, 122, 139, 140) that do not appear in any of the source trees; all of these occur towards the tips of the tree. This is a general problem in supertree construction and such clades should be collapsed as they have no support [41]. To demonstrate where the MRP method has performed badly we have included the novel clades in all figures and list details in the figure legends. In addition, 58 nodes are supported by only one character (see additional file 1). Each of these nodes is left over from a single source tree. Assessing the support for such nodes is problematic because this may simply reflect a

lack of research directed at the taxa in question. A major challenge for supertree construction is to develop measures of support that reflect the robustness of nodes in the source trees. We list the number of characters supporting each node (additional file 1) but stress that these are not measures of tree robustness and may not be directly comparable even within the same tree. This is because the taxon coverage across source trees is highly variable so some nodes have more potential support than others. Furthermore, because measures of support used in the source trees differ between studies (some source trees include no measures of support), it is impractical and of dubious value to use these measures to assess the robustness of the supertree.

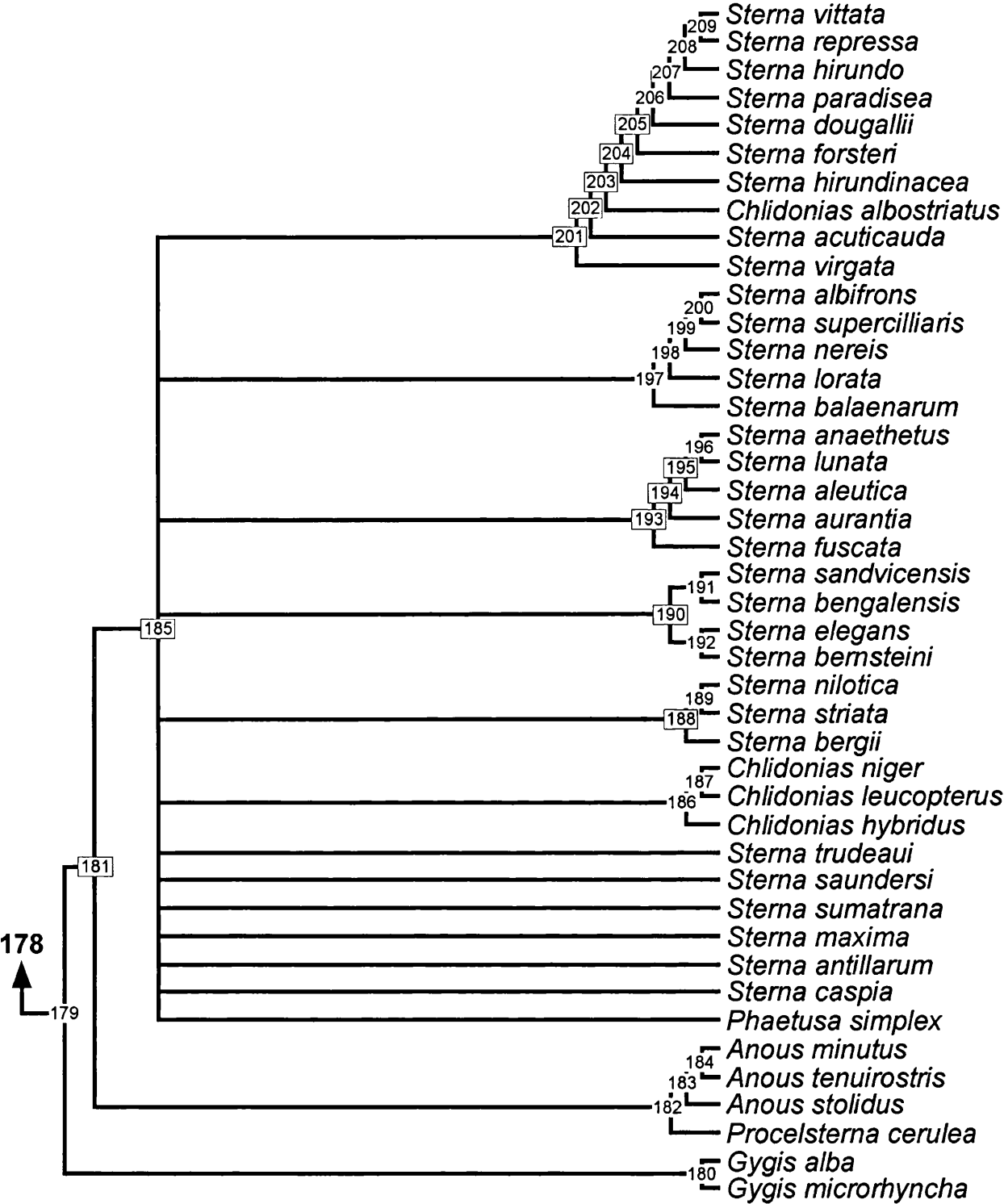
The majority of unresolved nodes in the shorebird supertree are located towards the tips of the phylogeny. For example, the genus *Gallinago* forms a monophyletic clade but only two pairs of species are resolved from 14 species (*G. megala* and *G. negripennis*; *G. macrodactyla* and *G. media*) in the majority-rule tree. Only the latter relationship remains in the strict consensus tree. In addition, clades including the genera *Charadrius* and *Vanellus*, *Calidris* and *Tringa*, *Sterna*, and *Scolopax* are poorly resolved. This may reflect a bias in phylogenetic studies of shorebirds. For instance, we found six source trees for *Alcinae* [43-48] but none devoted to *Scolopax* or *Gallinago*. Thomas *et al.* [49] indicate that this may be a problem for



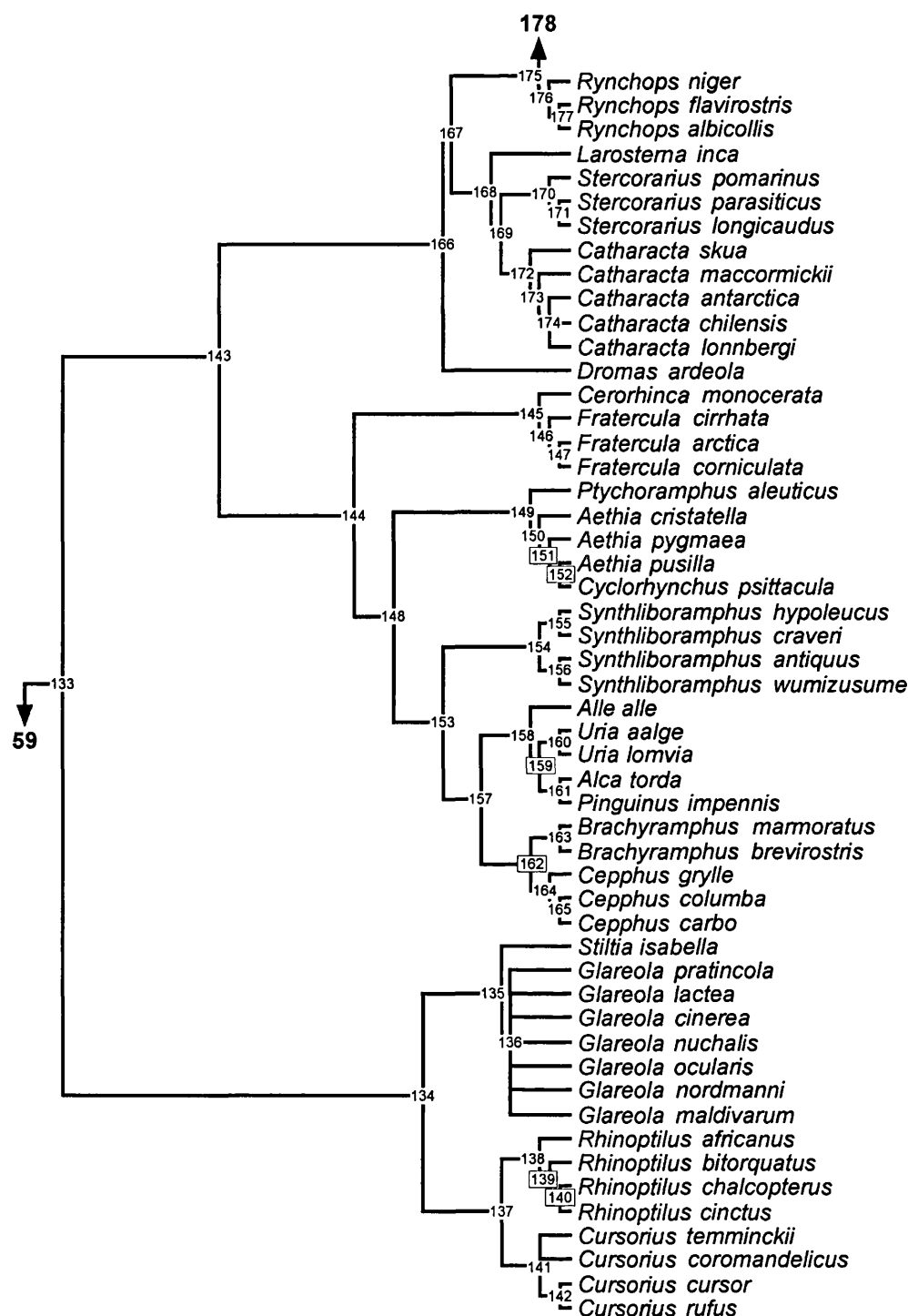
**Figure 2**  
**Summary of shorebird supertree.** Family and subfamily level relationships of shorebirds based on 50% majority rule tree. Numbers on nodes refer to age estimates in additional file 1. Boxed node numbers indicate that node collapses to its immediate ancestor in the strict consensus tree (see also additional files 2 and 3 for the full 50% majority rule and strict consensus trees respectively).



**Figure 3**  
**Phylogeny of Larini.** 50% majority rule supertree showing the relationships of the Larini. Numbers on nodes refer to age estimates in additional file 1. Boxed node numbers indicate that node collapses to its immediate ancestor in the strict consensus tree (see also additional files 2 and 3 for the full 50% majority rule and strict consensus trees respectively).



**Figure 4**  
**Phylogeny of Sternini.** 50% majority rule supertree showing the relationships of the Sternini. Numbers on nodes refer to age estimates in additional file 1. Boxed node numbers indicate that node collapses to its immediate ancestor in the strict consensus tree (see also additional files 2 and 3 for the full 50% majority rule and strict consensus trees respectively).

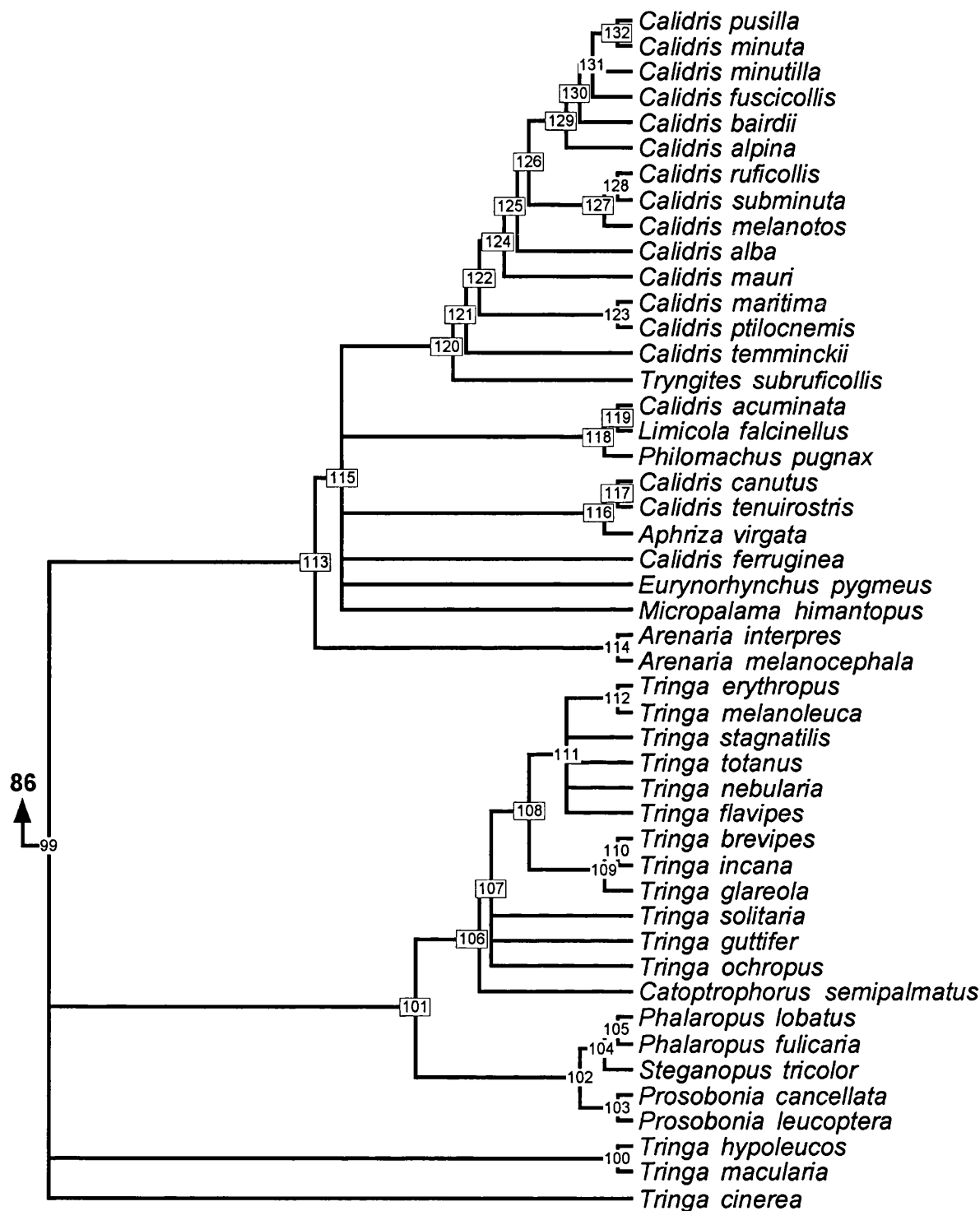


**Figure 5**  
**Phylogeny of Rynchopini, Stercorariini, Dromas, Alcinae, and Glareolidae** 50% majority rule supertree showing the relationships of the Rynchopini, Stercorariini, Dromas, Alcinae, and Glareolidae. Numbers on nodes refer to age estimates in additional file 1. Boxed node numbers indicate that node collapses to its immediate ancestor in the strict consensus tree (see also additional files 2 and 3 for the full 50% majority rule and strict consensus trees respectively). Node numbers 139 and 140 have no support from any source tree and are novel clades.

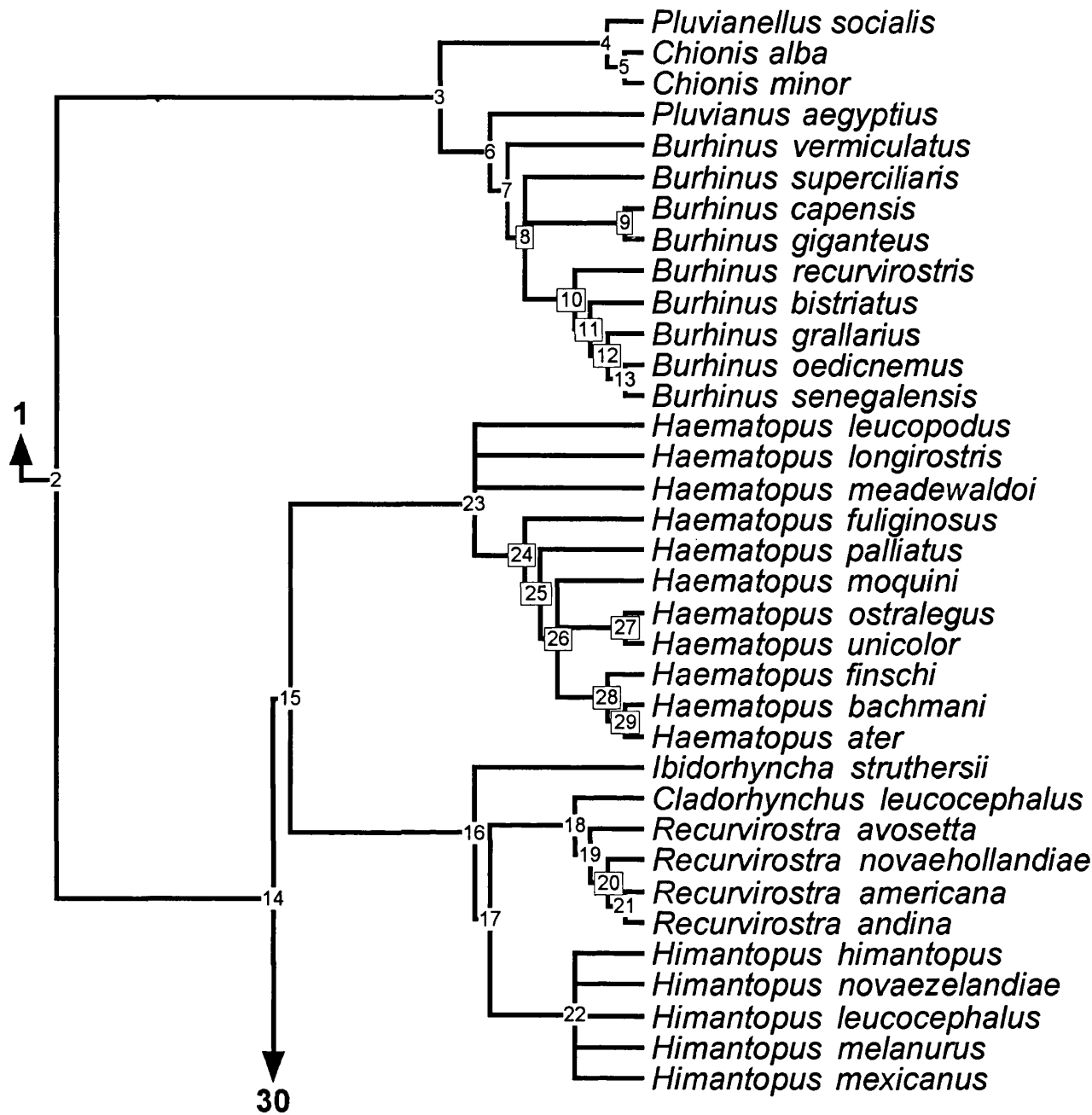


**Figure 6**  
**Phylogeny of Jacanidae, Rostratulidae, Thinocoridae, Pedionomidae and Scolopacidae** 50% majority rule super-tree showing the relationships of the Jacanidae, Rostratulidae, Thinocoridae, Pedionomidae and Scolopacidae. Numbers on nodes refer to age estimates in additional file 1. Boxed node numbers indicate that node collapses to its immediate ancestor in the strict consensus tree (see also additional files 2 and 3 for the full 50% majority rule and strict consensus trees respectively). Node numbers 85 and 89 have no support from any source tree and are novel clades.





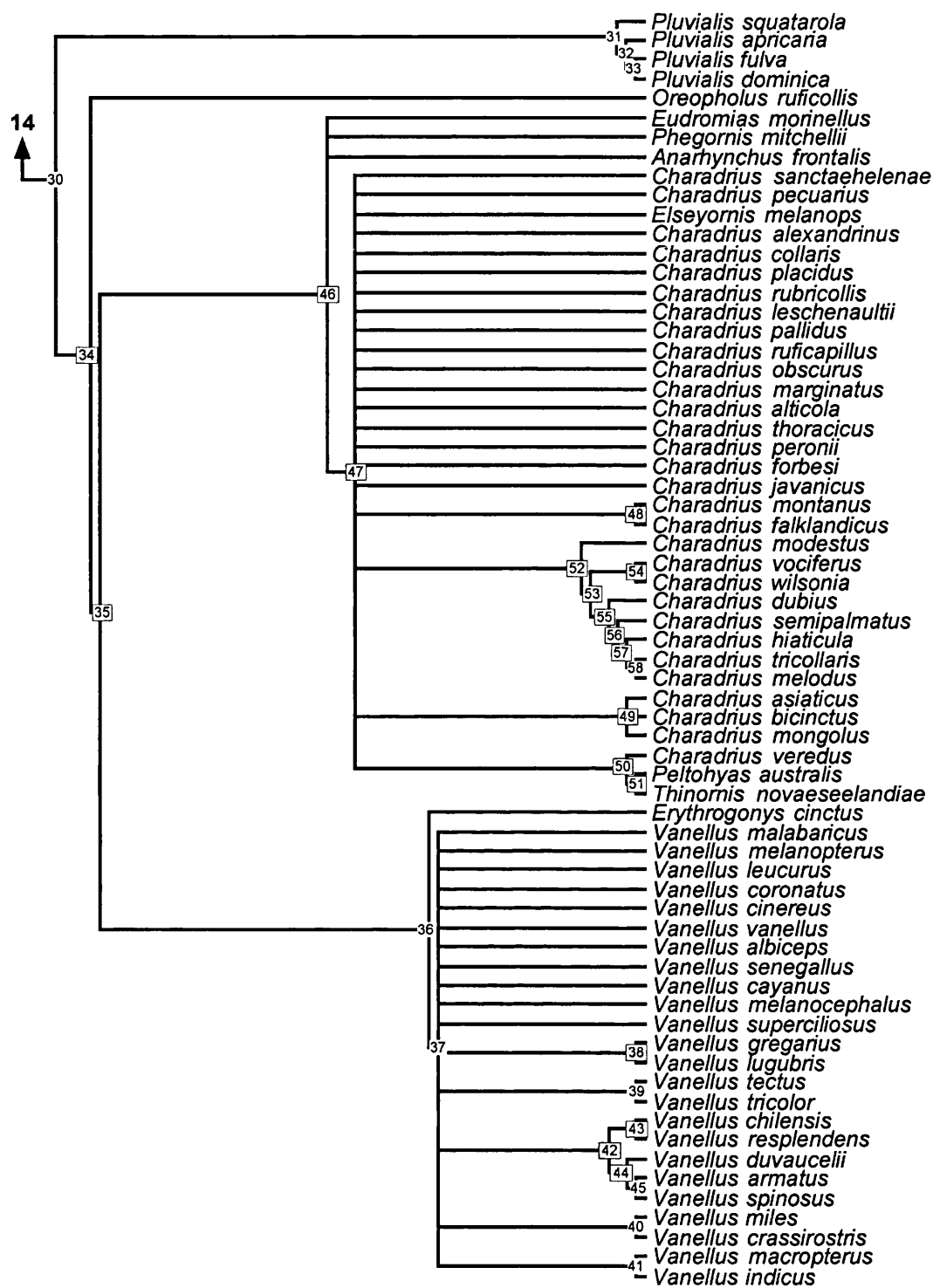
**Figure 7**  
**Phylogeny of Scolopacidae** 50% majority rule supertree showing the relationships of the Scolopacidae. Numbers on nodes refer to age estimates in additional file 1. Boxed node numbers indicate that node collapses to its immediate ancestor in the strict consensus tree (see also additional files 2 and 3 for the full 50% majority rule and strict consensus trees respectively). Node numbers 108 and 122 have no support from any source tree and are novel clades.



**Figure 8**  
**Phylogeny of Pluvianellidae, Chionidae, Burhinidae, Haematopodini and Recurvirostrini** 50% majority rule super-tree showing the relationships of the Pluvianellidae, Chionidae, Burhinidae, Haematopodini and Recurvirostrini. Numbers on nodes refer to age estimates in additional file 1. Boxed node numbers indicate that node collapses to its immediate ancestor in the strict consensus tree (see also additional files 2 and 3 for the full 50% majority rule and strict consensus trees respectively). Node numbers 20 and 29 have no support from any source tree and are novel clades.

shorebird studies in general and reported a strong skew favouring research on northern hemisphere species.

In contrast to the within genera relationships, the generic and family levels are generally well resolved. The supertree



**Figure 9**  
**Phylogeny Charadriiinae** 50% majority rule supertree showing the relationships of the Charadriiinae. Numbers on nodes refer to age estimates in additional file 1. Boxed node numbers indicate that node collapses to its immediate ancestor in the strict consensus tree (see also additional files 2 and 3 for the full 50% majority rule and strict consensus trees respectively). Node number 57 have no support from any source tree and are novel clades.

indicates three monophyletic Charadriiformes lineages (figure 2). Family and subfamily resolution within each lineage is high, however the relative position of each group is unresolved in the strict consensus tree. This is an important point because the deepest relationships of shorebird phylogeny are contentious [22]. The 50% majority-rule consensus tree indicates that the gulls and allies (Larini, Sternini, Rynchopini, Stercorariini, *Dromas*, Alcinae, and Glareolidae) are sister to the sandpipers and allies (Scolopacidae, Jacanidae, Rostratulidae, Thinocoridae, Pedionomidae). The most basal lineage includes the plovers and allies (Charadriinae, Pluvianellidae, Chionidae, Burhinidae, Haematopodini and Recurvirostrini). The gulls and allies clade is most consistent with DNA-DNA hybridisation [16], indicating that Larini are sister to Sternini and that Rynchopini are sister to this group. This conflicts with morphology-based topologies where Stercorariini are sister to Larini and Sternini with Rynchopini basal to both. Indeed, the position of Stercorariini remains controversial and most recently they were placed as sister to Alcinae [20-22]. In contrast, morphological evidence [18,19] places Alcinae at the base of the whole Charadriiformes tree with Stercorariini sister to Larini. Thus, the position of Alcinae is uncertain and appears to be dependent on the type of data, with fundamental differences between molecular based analyses and morphological analyses. The taxon sampling of previous morphological and molecular studies varies considerably and it may be this, rather than genuine differences in the phylogenetic signal of different data types, that is the cause of conflict in resolving the phylogenetic position of Alcinae. However, it is encouraging that van Tuinen *et al.* [17] suggested that new unpublished osteological data are consistent with the more derived position indicated by molecular data. The supertree resolves Glareolidae outside the Larini, Sternini, Rynchopini, Stercorariini, *Dromas*, Alcinae clade. This is also the case with recent molecular and previous DNA-DNA hybridisation studies. Morphological studies have failed to resolve the position of Glareolidae, placing the family in a large polytomy with all other major groups except Alcinae and the sandpipers and allies (fig. 1). A novel development in shorebird phylogeny is the placement of the black-rumped buttonquail *Turnix hottentotta* as a sister to the gulls and allies (Larini, Sternini, Rynchopini, Stercorariini, *Dromas*, Alcinae, and Glareolidae) based on the nuclear RAG-1 gene [20]. We did not include this species in the supertree because to date Paton *et al.* [20] remains the only study to reveal an apparently robust relationship. More diverse sampling of the buttonquails (Turnicidae) is essential to corroborate the general affinities of this family.

The relationships within the plover clade appear to be reasonably stable. Morphological, molecular, and DNA-DNA hybridisation all place Charadriinae as sister to Haemat-

opodini and Recurvirostrini; our supertree is consistent with these relationships. However, it is not clear whether Burhinidae and Chionidae are sister to each other [20-22] or whether Chionidae are sister to a Charadriinae, Haematopodini, Recurvirostrini, and Burhinidae clade [16]. Our supertree also included Pluvianellidae, a family consisting of only one species (magellanic plover *Pluvianellus socialis*) and places this as sister to Chionidae. If Pluvianellidae are excluded, the supertree is consistent with the sister group relationship of Burhinidae and Chionidae.

The sister group relationship of Jacanidae to Rostratulidae is well established [16,18-22] and is found in our supertree. The supertree resolves the Thinocoridae and Pedionomidae as sister taxa and this group is sister to the Jacanidae and Rostratulidae. The large Scolopacidae clade is at the base of the sandpiper clade consistent with recent molecular studies [20-22] and the DNA-DNA hybridisation tapestry [16].

Taken together, it is evident that the supertree is generally more consistent with molecular data (both recent sequence studies and DNA-DNA hybridisation) than with analyses based on morphology. However, it is of course possible that this reflects the greater number of molecular source trees available rather than indicating that molecular data is actually better at resolving shorebird phylogeny. We included several large morphological phylogenies [e.g. [18,19,26,30,43]] but the majority of source trees (29 out of 51) were based on molecular evidence (see additional file 5).

#### Node dates

The higher resolution of the majority-rule tree means it is more likely to be of use in comparative studies. We therefore estimated node ages for this topology only (see additional file 1 and 2). We stress that our estimates of node dates are a first attempt at dating the whole tree and have several limitations. First, the fossils used to calibrate seven nodes in the tree are unlikely to be the earliest members of their respective families thus these dates will be underestimates. Second, we assumed that the fossils are grouped with the extant members of the family but this requires formal testing in a phylogenetic framework. Third, the pure birth model assumes that no extinction occurs but this may be unrealistic and it is likely that extinction processes have reduced the representation of older lineages [15]. Furthermore, this model is derived from the topological structure of the tree so errors in tree reconstruction will likely lead to errors in branch length estimation. However, this approach has been employed previously in supertrees of primates [39] and carnivores [15] explicitly to facilitate comparative analyses. Despite these caveats, simulation studies have demonstrated that comparative methods such as independent contrasts are

robust to errors in branch length [50] and no viable alternative for dating supertrees has been proposed. Nonetheless, we urge that alternative branch length assumptions are explored if the shorebird supertree is used in future comparative studies. At present, the calibrated RAG-1 tree of Paton *et al.* [20] remains arguably the most thorough and reliable measure of divergence times for Charadriiformes.

A fuller understanding of the phylogenetic affinities of fossil shorebirds will probably improve estimates of node ages for the group. For example, the extinct form Graculavidae, is represented by fossils from the Maastrichtian of New Jersey [51] and Cretaceous of Wyoming [52] but its position within the shorebird clade is unclear. Feduccia [53] suggests that it may be basal and a formal corroboration of this would support proposals for a late Cretaceous origin of shorebirds. The difficulties in dating the shorebird tree are further illustrated by fossil representatives of Recurvirostrini and Burhinidae which are much older than current estimates suggests. The earliest record of the Recurvirostrini is estimated to be over 50 million years old [54] whilst recent discoveries of a possible member of the Burhinidae are dated to around 70 mya [55,56]. There is clearly a need for an integrated phylogenetic study including both extinct and extant shorebirds.

#### Supertree bias

Supertrees are still at an early stage of development and many aspects of MRP, and supertree methods in general, are not yet clearly understood. Steps can be taken to ensure that the supertree includes the most appropriate sets of sources trees, such as only using trees from explicitly phylogenetic studies. This is not always straightforward and could result in the exclusion of important information. For instance, in our shorebird supertree, we included Sibley and Ahlquist's DNA-DNA hybridisation tapestry [16] although this is based on distance measures rather than more rigorous phylogenetic methods. Even if very strict tree selection criteria are applied, there are still likely to be biases in the data set. For example, not all source trees are equally well supported, yet in most supertree analyses each tree is treated equally [57]. This is a problem for supertree construction because whilst it is theoretically possible, and indeed beneficial, to weight source trees based on support values [57] it is rarely possible in practice. Many source trees do not have support values and those that do may use different methods, (e.g. bootstrapping or decay indices) which cannot be directly compared with each other. An additional problem that has not been fully resolved relates to correlations between source trees [58]. Several source trees based on the same data set may unduly increase the influence of that data set on the supertree analysis. However, there is no formal way of determining how much overlap to allow and the choice

of source trees that go into supertree construction inevitably involves some degree of subjective reasoning. For the shorebird supertree we used strict Reduced Cladistic Consensus trees to summarise potential source trees that were from the same data set but based on different methods. For example, Thomas *et al.* [22] based their phylogeny on cytochrome-*b* but used a range of methods including parsimony and Bayesian analyses. We therefore combined these trees to minimise bias. In contrast, Ericson *et al.* [21] used two types of data: sequences from the nuclear RAG 1 gene and sequences from the myoglobin intron II. They carried out three analyses: each gene separately and then the two combined in a single analysis. In this case, we used three source trees. It could be argued that the combined analysis of Ericson *et al.* [21] should be excluded because of the possible overlap with the individual analyses. However, under the principle of total evidence, the combined data set may result in novel relationships being revealed [31,33] and therefore could contribute important information to the supertree. Simulation and empirical studies are required to fully understand these and other possible biases in supertree construction (e.g., the influence of source tree size and shape) and formal protocols for the selection of source trees are desirable. For transparency, we include a summary of the source trees used, data type, and the main taxa included in the study (additional file 5).

Our shorebird supertree is highly consistent with recent advances in the molecular phylogenetics Charadriiformes. However, we urge caution when using the tree in comparative analyses and encourage the additional use of alternative phylogenies and branch length assumptions. It is particularly important to note that the position of some groups such as the Alcinae remains controversial and that although the majority rule tree is consistent with recent molecular studies, the strict consensus tree fails to resolve the deepest nodes.

#### Conclusions

The supertree presented here is, to our knowledge, the first attempt to reconstruct the phylogeny of the entire order Charadriiformes. Overall, the supertree is highly consistent with recent molecular hypotheses of shorebird phylogeny. However, it is apparent that fresh attempts to resolve both the phylogeny and estimates of age will be dependent on further gene sequencing and new fossil discoveries. The affinities of the Alcinae and the relationships between the three major shorebird clades require further corroboration, and studies of several genera such as *Gallinago* and *Vanellus* are desirable. Furthermore, additional work is required to establish the true affinities of the Turnicidae. Nonetheless, it appears that shorebird phylogeny is gradually approaching a consensus view. The broad taxonomic scope and consistency of the supertree mean that

is of potentially great value to future comparative studies (accepting the caveats discussed above) of the behaviour, life-history, ecology and conservation of this diverse group.

## Methods

### Supertree construction

Possible source trees were identified from online searches of Web of Science <http://wos.mimas.ac.uk> covering the years 1981 to 2004. We used the single key strings phylogen\*, cladistic\*, clado\*, classif\*, systematic\*, and taxonom\* (where the asterisks allow variations such as "phylogeny" or "phylogenetics") in the topic field, in conjunction with a major Charadriiformes taxon name (scientific or common). As supertree methods have been criticized for being biased towards historical trends, we preferred those studies that explicitly set out to derive a phylogenetic hypothesis and so exclude purely (and typically older) descriptive taxonomic works. The Sibley and Ahlquist [16] DNA-DNA hybridisation tapestry may be viewed as non-cladistic, but it was clearly the authors' intention to reconstruct the phylogeny of birds. Furthermore, it provided a vital catalyst for subsequent studies of avian (including shorebird) phylogeny. We therefore included the DNA-DNA hybridisation hypothesis as a source tree in our analyses. Simulation studies have demonstrated that the performance of supertree methods is improved by including at least one taxonomically complete (or near complete) source tree [57]. We therefore make an exception to our self-imposed rule, and in addition use the taxonomic hierarchy of Monroe and Sibley [1] as a source tree as this includes all extant Charadriiformes species. We acknowledge that this taxonomy is based largely on Sibley and Ahlquist's [16] DNA-DNA hybridisation tapestry.

The initial search identified 78 source trees from 44 publications. Each source tree was typed as a text file in Nexus format [59]. We coded trees to the species level with species names taken from Monroe and Sibley [1], but note that *contra* Monroe and Sibley [1], we use Charadriiformes not Charadrii to refer to the whole group. Several studies included the gull *Larus thayeri* [26,60-63] either as a subspecies of *Larus glaucooides* (*Larus glaucooides thayeri* in Monroe and Sibley [1]) or a species in its own right. In recognition of this, we included *Larus glaucooides thayeri* as the only subspecies in our data set thus increasing the total taxa to 366. Monroe and Sibley [1] include 16 species of the family Pteroclididae within the Charadriiformes. However, the relationship of this family to the Charadriiformes is uncertain and they have recently been placed in their own order [64]. We include the Pteroclididae in our analyses only as a means of rooting the tree. Where there were multiple most parsimonious trees (MPTs), or where source trees had been derived from predominantly

overlapping data (e.g., from the same data but using alternative methods), we used RadCon [65] to produce strict Reduced Cladistic Consensus trees (RCC [66,67]). The output is in the form of a reduced consensus profile and from this we selected the tree with the highest Cladistic Information Content (CIC) [65,68]. This resulted in a total of 51 source trees from which our supertree is derived and these are summarised in additional file 5.

We produced an MRP matrix of the 51 Nexus [59] source trees in RadCon [65] (see additional file 6 for the MRP file). We used the original MRP coding method of Baum [37] and Ragan [38]. Weighting source trees based on node support such as bootstrapping improves the accuracy of MRP supertrees [57]. However, this is only possible if all source trees can be weighted on the same criteria [57]. The absence of branch support measures in many of the shorebird source trees precludes this approach from the present study; hence, subsequent analyses were conducted using equally weighted parsimony.

The tendency of large data sets to produce many sub-optimal trees that are close in length and topology to the shortest tree is a serious problem in phylogenetics. Standard heuristic searches frequently are trapped searching within globally sub-optimal "islands" and the tree search is often aborted before completion. Nixon [69] proposed a new method to avoid this problem. The "Parsimony Ratchet" reweights a random set of characters from the data set. This may result in the tree island no longer representing a local optimum and the heuristic search continues until a new optimum is reached. The algorithm then reverts to the original weighting and the search continues. Nixon [69] demonstrated the efficacy of the method on a 500-taxon data set, where the ratchet-based search found a tree two steps shorter than standard heuristic searches. We used PAUPRat [70] to implement a parsimony ratchet in PAUP\* [59]. The default settings of 200 iterations and 15% perturbation of characters for reweighting were used and we carried out 20 replicates. Equally parsimonious trees were summarized using both strict and 50% majority-rule consensus methods.

We did not calculate any measures of branch support for two reasons. First, their validity and meaning is questionable in MRP supertrees [41]. Second, the number of taxa included in our data set is too large to allow practical calculation of any branch support indices (e.g., decay indices [71]) on a desktop computer.

### Dating the supertree

Following Purvis [39] and Bininda-Emonds *et al.* [15] we dated the supertree using both absolute and relative dates. We used data from the Fossil Record 2 [54] as the source of fossil-based absolute dates. This yielded estimates for

Jacaniidae (*Nupharanassa tolutaria*, Rupellian), *Phalaropus* (*Phalaropus elenora*, Middle Pliocene), Burhinidae (*Burhinus lucorum*, Lower Miocene), Glareolidae (*Paractiornis perpusillus*, Lower Miocene), Alcinae (*Petralca austriaca*, Rupellian), Stercorariini (*Stercorarius* sp., Middle Miocene), and Larini (undetermined, Rupellian). We took the midpoint of the range from the Fossil Record 2 [54] as our date estimate. More recent publications of fossil Charadriiformes were not included because they either represent specimens that are younger or have not been assigned to families that are represented amongst the extant Charadriiformes (such as Turnipacidae [72]). We assumed that fossil dates represent the earliest occurrence for each group which inevitably resulted in underestimates of clade age. The fossil record of Charadriiformes is amongst the best of the modern bird groups [17] in terms of the numbers of taxa, but many specimens are fragmentary and reliable estimates of divergence dates are dependent on a limited number of exceptional specimens [73]. The phylogenetic affinities of the fossil shorebirds in relation to their extant relatives have not yet been fully established, hence have implicitly assumed that fossil representatives of extant groups would be resolved amongst their living relatives.

Source trees may include estimates of relative branch lengths (e.g., genetic distances). This allows further dating of the supertree but is problematic because different relative estimates are not comparable and cannot be applied directly to the supertree [39]. However, where a source tree shares a node that has an absolute date in the supertree (a node dated from fossil evidence), the relative branch lengths can easily be converted to estimates of age. All taxa in our supertree are either extant, or very recently extinct; hence, the tips of the calibrated supertree should be equidistant from the root of the tree. In source trees where the relative branch lengths are not equidistant from the root, we followed the protocol of Purvis [[39]; p.407–8]. We estimated relative dates using the local molecular clock logic [74] as implemented by Purvis [39] and Bininda-Emonds *et al.* [15]. For example, consider three taxa A, B, and C where A and B are sister taxa and C is sister to A and B. The root is dated to 10 million years (myr) from fossil evidence, and independent molecular data provides estimates of divergence based on the number of substitutions per site. The molecular estimates of branch lengths are as follows: A, 6 substitutions; B, 8 substitutions; C, 20 substitutions; A and B are 11 substitutions from the root. A and B are therefore separated from their common node by a mean of 7 substitutions. The total length from A and B to the root is thus 18 substitutions compared to 20 for C (a mean of 19). This can be converted to date estimates such that 19 substitutions are equivalent to 10 myr. The dates of the tree are then: ((A: 3.68, B: 3.68), C: 10)). There were no cases where multiple

source trees with molecular divergence dates were able to provide estimates for the same node. We estimated relative dates from multiple nodes rather than a single dated node to minimise correlative errors in estimates.

To provide date estimates for all nodes in the tree we employed a pure birth model to date nodes for which absolute and relative dates could not be attained [39]. Pure birth models infer that a clade's age is proportional to the logarithm of the number of species within the clade:

date of daughter = date of ancestor \* (log daughter clade size / log parent clade size)

For example, the age of a daughter node that subtends 12 taxa, estimated from its immediate ancestor dated to 20 myr and which subtends 19 taxa is:

$$20 * (\log(12) / \log(19)) = 16.879$$

We applied this approach to estimate the ages of daughter nodes based on dates (absolute or calibrated) of ancestral nodes. We had no ancestral node on which to base estimates of the most basal clade. In this case, we rearranged the pure birth formula and calculated the age of the ancestral node from its two daughter nodes, taking the mean as our "best estimate". Finally, to estimate the ages of nodes between daughter and ancestor nodes of known age we spaced the nodes evenly along the branches length [75].

### Authors' contributions

GHT assisted in the design of the study, carried out the phylogenetic analyses and node dating, and drafted the manuscript in partial fulfillment of a doctoral degree at the University of Bath. MAW assisted in the design of the study and with editing and revision of the manuscript. TS assisted in the design of the study, collection of source trees, and editing and revision of the manuscript. All authors read and approved the final manuscript.

### Additional material

#### Additional File 1

*Estimates of node ages and node support (branch lengths.xls)* Node numbers correspond to figures 2-9. Five types of estimate were used: a) absolute dates from the fossil record; b) absolute dates from molecular point estimates; c) relative dates based on branch length estimates from molecular studies; d) estimates based on a pure birth model (see text for details); and e) even spacing of nodes along branches with daughters and ancestors of known age. The numbers of characters supporting each node are provided (column D), this is equivalent to the number of source trees that share the equivalent node (see text for details).

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[<http://www.biomedcentral.com/content/supplementary/1471-2148-4-28-S1.xls>]

### Additional File 2

**Shorebird supertree (50% majority-rule consensus; majrulesupertree.tiff)** Shorebird supertree based on 50% majority-rule consensus of 1496 shortest trees with calibrated branch lengths. Scale bar indicates time from the present in millions of years.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-2148-4-28-S2.tiff>]

### Additional File 3

**Shorebird supertree (strict consensus; strictsupertree.tif)** Shorebird supertree based on 50% majority-rule consensus of 1496 shortest trees.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-2148-4-28-S3.tif>]

### Additional File 5

**Source trees (source trees.xls)** A summary of each tree used is given including the data type and main taxa studied. This is a brief summary and the original papers should be consulted for full details.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-2148-4-28-S5.xls>]

### Additional File 6

**MRP matrix (shorebirdMRP.txt)** The MRP matrix used in the shorebird supertree analysis.

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[<http://www.biomedcentral.com/content/supplementary/1471-2148-4-28-S6.txt>]

### Additional File 4

**Calibrated supertree (shorebirdsuptree.txt)** The supertree in nexus format including branch length estimates.

Click here for file

[<http://www.biomedcentral.com/content/supplementary/1471-2148-4-28-S4.txt>]

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**Chapter 4.** *Developmental mode constrains rates of phenotypic evolution of sexual conflict, life-histories and ecology in shorebirds*

Gavin H. Thomas, Robert P. Freckleton, and Tamás Székely

## **Abstract**

Does offspring demand constrain the evolution of traits associated with sexual conflict, life-histories, and ecology? Rates of evolution are unlikely to be constant across all lineages, and rates of evolution of some traits may be influenced by others. The developmental mode of the young is expected to influence rates of phenotypic evolution because semiprecocial young place demands on the parents in terms of food provisioning whereas precocial offspring do not. Using a novel maximum likelihood method we compare rates of evolution between precocial and semiprecocial members of the avian order Charadriiformes (shorebirds, gulls and alcids). First, our results show that rates of evolution of mating and parental behaviour, and sexual size dimorphism are higher amongst species with precocial young than those with semiprecocial young. Second, body size has diverged more rapidly amongst semiprecocial taxa than precocial taxa, but adult mortality shows the opposite trend. Third, precocial species have diverged more rapidly than semiprecocial species in habitat quality and bill length dimorphism. Taken together, our results show that the demands of the young do influence rates of phenotypic evolution of traits associated with sexual conflict, life-histories, and ecology.

## 1. INTRODUCTION

Why are some lineages phenotypically diverse whilst others are conservative? Selection uses whatever variation is available (Gould & Lewontin 1979) and it is likely that the expression of some phenotypic traits depends on the state of others (Wilson 1975). Techniques have been proposed to: compare rates of phenotypic evolution against a neutral model of evolution within a lineage (Lynch 1990; Martins 1994); compare rates of evolution among monophyletic clades (Garland 1992); and to investigate rates of correlated character change (Felsenstein 1985; Harvey & Pagel 1991; Pagel 1994, 1997). For example, ungulates have higher rates of evolution of limb proportions than carnivores (Garland 1992; Garland & Janis 1993); and, rates of nucleotide substitution in nuclear DNA increased in fungi species after a transition towards mutualism (Lutzoni & Pagel 1997). Here, we focus on the problem of comparing rates of evolution between clades that differ in the expression of a discrete, binary phenotypic trait.

In birds, the morphological, physiological, and behavioural state of the chicks at hatching (developmental mode) ranges from naked and helpless (altricial; e.g. passerines) to down-covered and independent (precocial; gamebirds, many shorebirds). An important division along this continuum occurs between semiprecocial species, in which the chicks are dependent on the parents for food, and precocial species, in which the chicks feed themselves within hours of hatching. There are no major morphological or physiological differences between semiprecocial and precocial chicks (Starck & Ricklefs 1998a). In this study we ask if the state of development of hatchlings influences the evolution of traits associated with sexual conflict, life-histories, and ecology.

### **(a) *Sexual conflict***

We would expect the mode of chick development to influence sexual conflict because precocial development emancipates the parents from food provisioning. In semiprecocial species offspring demands are high, and there are associated costs in offspring survival if one parent deserts. Thus, semiprecocial taxa are likely to be restricted to biparental care and social monogamy (Orians 1969; Temrin & Tullberg 1995; Ligon 1999; Bennett & Owens 2002). In contrast, in precocial species, where the demands of the offspring are small, the amount of parental care required is likely to be

reduced with low costs of offspring survival if one parent deserts. The consequence of this is that in precocial lineages there exists the potential for a wide range of parental and mating strategies and the divergence of mating optima between the sexes (Székely *et al.* 1996; Orians 1969; Temrin & Tullberg 1995; Bennett & Owens 2002). Divergence of mating optima drives sexual conflict (Chapman *et al.* 2003). We therefore predict that the rate of diversification of parental care, social mating system, and of traits associated with sexual conflict will be higher in precocial species than in semiprecocial species.

#### **(b) *Life-histories***

The state of development of hatchlings has been argued to be key to explaining variation in life history traits in birds (Starck & Ricklefs 1998b). Because the parents must feed the chicks in less developed species, the maximum rate of reproduction is relatively low. More generally, since the need to feed the chicks is fully relaxed in precocial species, the reproductive rate is no longer dependent on the ability of the parents to gather food (Starck & Ricklefs 1998b) and this may lead to low variation in clutch size (Gillespie 1974). In contrast, fecundity is dependent on the ability of the parents to feed the chicks in semiprecocial taxa (Gillespie 1974). In such species, food provisioning is likely to be limited by extrinsic factors such as climate and predation risk. Thus, fecundity is expected to vary in semiprecocial taxa with these extrinsic factors but not in precocial taxa. Furthermore, since life-history traits may be tightly related to one another (Stearns 1992), with altriciality associated with slow life histories (slow development, low fecundity, low mortality), and precociality associated with fast life histories (fast development, high fecundity, high mortality; Bennett & Owens 2002), we extend the prediction of reduced life-history variation in precocial taxa to life-histories in general.

#### **(c) *Ecology***

We would expect mode of development of offspring to be associated with ecological requirements and tolerance. Precocial chicks exhibit a high degree of thermal independence (Visser 1998) and as a consequence precocial development is likely to be associated with ecological flexibility (Starck & Ricklefs 1998b). We therefore predict that precocial species will vary more ecologically (e.g. breeding habitat, breeding latitude, feeding mechanism) than semiprecocial species.

The avian order Charadriiformes (sandpipers, plovers, gulls, auks and allies; shorebirds hereafter) is amongst the most diverse of all birds and makes an excellent model system to investigate the influence of developmental mode on phenotypic evolution. First, shorebirds can be divided into precocial taxa (plovers, lapwings, sandpipers, jacanas) and semiprecocial taxa (oystercatchers, stone curlews, gulls, alcids). Second, the range of parental care strategies and social mating systems are unsurpassed in birds (Pitelka *et al.* 1974; Erckmann 1983; Székely & Reynolds 1995; Reynolds & Székely 1997). Third, shorebirds breed worldwide in diverse ecological settings including temperate wetlands (e.g. oystercatchers), Arctic tundra (calidrine sandpipers), tropics (jacanas, painted-snipes), the Antarctic (sheathbills), and semi-desert (pratincoles and coursers). Finally, the availability of a supertree phylogeny (Thomas *et al.* 2004) including all extant members of the order provides an ideal phylogenetic framework for comparative studies.

Here, we describe a novel phylogenetic technique to compare rates of evolution in clades defined by a dichotomous trait based on Pagel's (1997) generalized least squares (GLS) method. We then use this method to compare rates of phenotypic evolution between precocial and semiprecocial shorebirds. Specifically, we investigate the influence of developmental mode on sexual conflict, life-histories and ecology.

## 2. MATERIALS AND METHODS

### (a) *Comparing rates of evolution*

Generalised least squares (GLS) is a statistical method that that can be applied to phylogenetic problems to control for non-independence among species by reference to a variance-covariance matrix of the expected similarity between species. (Pagel 1997; 1999). Under a Brownian model of character evolution, the amount of phenotypic change is expected to be proportional to time (Felsenstein 1985). Thus, the expected covariance of any two species is proportional to the sum of their shared branch lengths in the phylogeny.

We extend Pagel's (1999) model to test the null hypothesis of no difference in evolutionary rates in two parts of the phylogeny. We consider the case of differences

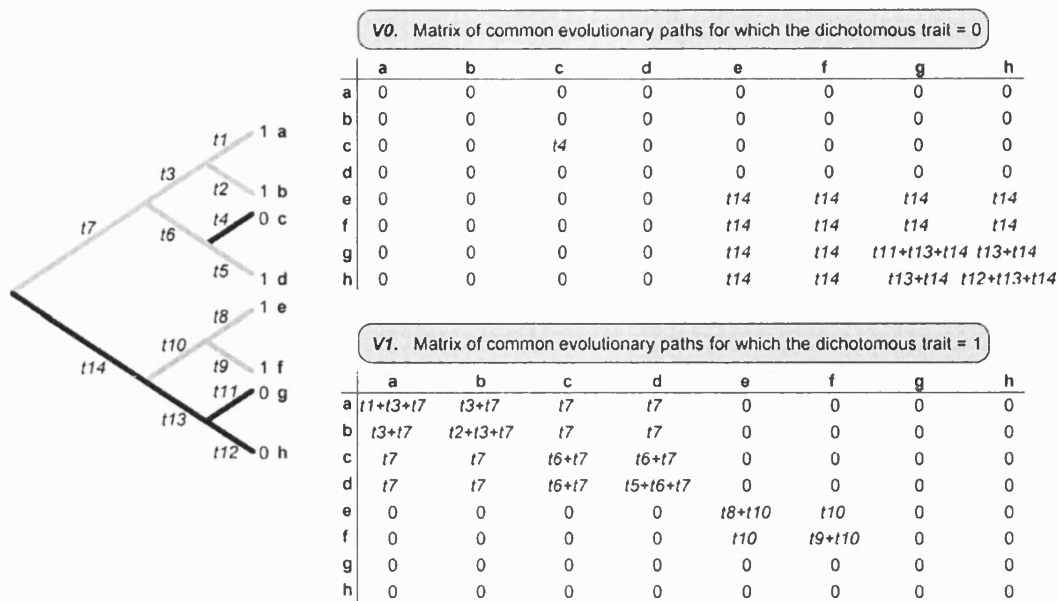
associated with the expression of a discrete, binary trait (states 0 and 1), although the method we describe could also be applied to the simpler case of a comparison of two monophyletic sister clades. A single variance-covariance matrix can only describe the covariance among species if we assume constant variance across the phylogeny (a single rate of trait evolution). Since our aim is to test for differences in rates of evolution in two parts of the phylogeny, two matrices are required; one matrix representing the expected covariance associated with the trait in state 0 (**V0**), and the other representing the expected covariance associated with the trait in state 1 (**V1**; see fig. 1 for an example). This requires the reconstruction of ancestral states for the binary trait in order to partition the phylogeny. Our method is applicable to any form of ancestral state reconstruction (e.g. maximum parsimony, maximum likelihood, Bayesian analysis). The standard, single-rate variance-covariance matrix, **V**, is given by the sum of **V0** and **V1**. If the null-model (constant Brownian variance across the phylogeny) is correct, then **V0** + **V1** will provide the best fit to the data and phylogeny. If the variance differs in the two parts of the phylogeny, then the matrices must be scaled accordingly. We introduce the parameter  $\theta$  such that  $\mathbf{V} = \mathbf{V0} + \theta\mathbf{V1}$ . The value of  $\theta$  is defined as the transformation of the phylogeny that makes the trait data best fit the Brownian motion model. The derivation and maximum likelihood estimation of  $\theta$  follows that for Pagel's (1999)  $\lambda$  (see Freckleton *et al.* 2002 for the full derivation of the likelihood model).

The estimate of  $\theta$  can be tested to determine whether the data fit the constant variance model or not. If  $L(\hat{\theta})$  is the log-likelihood at the maximum likelihood value of  $\theta$ , and  $L(\theta')$  is the log-likelihood at an alternative value of  $\theta'$ , then the log-likelihood ratio

$$\chi^2 = -2[L(\hat{\theta}) - L(\theta')]$$

will be asymptotically  $\chi^2$  distributed with one degree of freedom under the null hypothesis that  $\hat{\theta} = \theta'$ . A maximum likelihood value of  $\theta$  that does not differ significantly from 1 supports the (null) constant variance model. A value of  $\theta > 1$  can arise if there is greater variance in **V1** than in **V0** since the branch lengths (covariance) among matrix **V1** must be stretched to bring the two parts of the tree into common variance. A value of  $\theta < 1$  indicates the reverse.





**Figure 1.** Constructing variance-covariance matrices from a phylogeny. There are five species with trait 0, and three with trait 1. The branch lengths are shown as  $t_1$ ,  $t_2$ ,  $t_3$ ...etc. The first matrix (**V0**) defines paths for which the trait = 0 and contains all the shared path lengths for each species for which the trait = 0, otherwise the entries are zero. Similarly the second variance-covariance matrix (**V1**) contains all the shared path lengths for which trait = 1, otherwise the entries are zero. The usual variance-covariance matrix of the full phylogeny is the sum of these ( $V = V0 + V1$ ).

### (b) Data and phylogeny

We used the recent supertree phylogeny of shorebirds (Thomas *et al.* 2004) in all analyses because this is the only complete phylogeny available for the extant members of the group.

We collated a database of behavioural, morphological, life-history, and ecological traits of shorebirds from the literature to build on existing datasets (Székely & Reynolds, 1995; Reynolds & Székely, 1997; Székely *et al.*, 2000; Székely *et al.* 2004). Data on developmental mode from 203 species were extracted mainly from Thomas & Székely (submitted; see Chapter 5) and this was supplemented from the literature. Species were scored according to the feeding behaviour of hatchlings. Species in which the chicks fed themselves within hours of hatching were classed as precocial whereas species in which the chicks were reliant on the parents for feeding until fledging were classed as semiprecocial. Eight species begin life as semiprecocials but switch to precocial behaviour within one to two weeks of hatching (i.e. prior to fledgling; *Cursorius*

*coromandelicus*, *Cursorius cursor*, *Gallinago gallinago*, *Philomachus pugnax*, *Rostratula benghalensis*, *Scolopax minor*, *Scolopax rusticola*, *Xema sabini*). We repeated all of our analyses twice (see below), first with these eight taxa classified as precocial, and second with them classified as semiprecocial.

**Sexual conflict:** Male and female social mating system, and the duration of male and female parental care, were used as proxy measures of conflict over mating optima. Male social mating system was scored towards increased frequency of polygyny based on descriptions in the literature: 0, monogamous; 1, rare polygyny (<1% or limited anecdotal reports); 2, occasional polygyny (1-5%, polygyny is known to occur but it is infrequent); 3, moderate polygyny (6-20%, polygyny is well known but is not regarded as typical of the species); 4, frequent polygyny (>20%, polygyny is considered the main mating system for the species). Female social mating system was scored on the same scale but with respect to frequency of polyandry (from monogamy [0] to frequent polyandry [4]). The duration of care for each sex was scored following Székely & Reynolds (1995) and Reynolds & Székely (1997). Male displays are expected to reflect sexual selection with ground displays associated with male-male competition, and acrobatic aerial displays associated with female mate choice (Székely *et al.* 2000). Male display type was scored following Székely *et al.* (2000) and Székely *et al.* (2004). Sexual size dimorphism (SSD) in wing length was calculated as  $\log_{10}$  (male wing length / female wing length). Sexual size dimorphism (SSD) in body mass was calculated as  $\log_{10}$  (male body mass / female body mass). Note that we did not investigate rates of extra pair paternity because data for shorebirds is limited ( $n = 14$  species; Griffith *et al.* 2002).

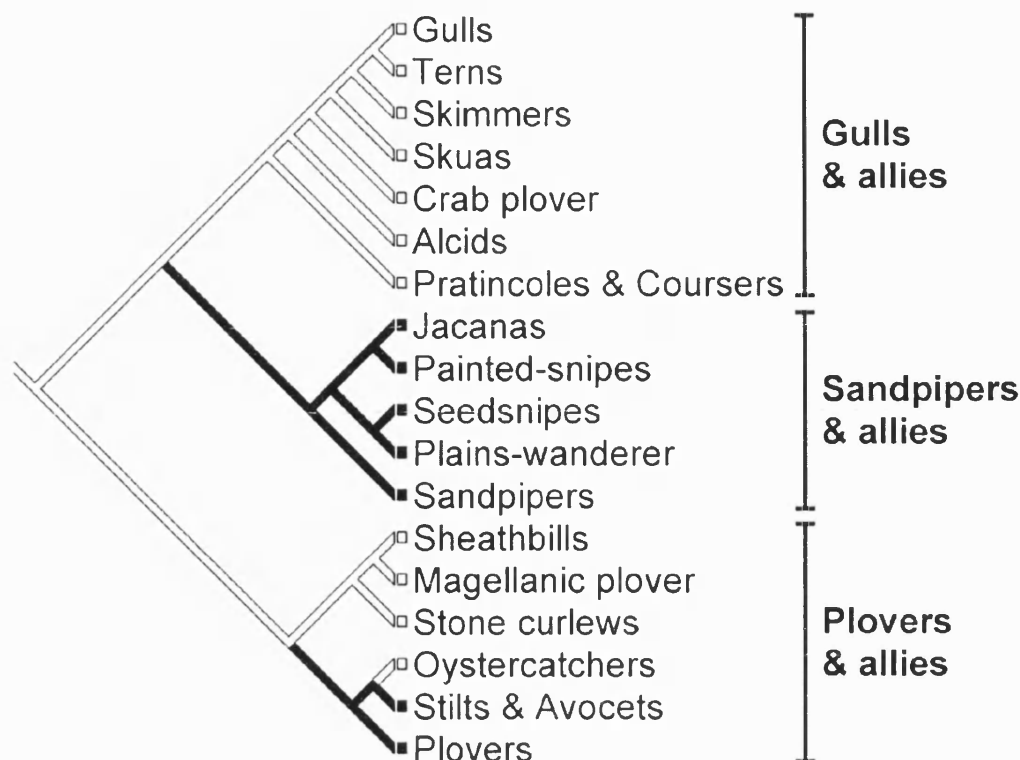
**Life-history:** We used the mean body size of males and females (measured using both body mass and wing length), clutch size (as a measure of fecundity), and adult mortality as measures of life-history variation. Large body size, small clutch size, and low mortality are associated with “slow” life-histories, whereas small body size, large clutch size, and high mortality are associated with “fast” life-histories (Bennett & Owens 2002).

**Ecology:** We used breeding habitat productivity, sexual dimorphism in bill length ( $\log_{10}$  [male bill length / female bill length]), and absolute breeding latitude (distance from the

equator to the centre of the breeding range in degrees) as measures of ecological diversity. Our breeding habitat scores follow Székely *et al.* (2004): 0, desert and semi-desert (low productivity); 1, dry grassland, tundra, dry forest (intermediate productivity); 2, wetland, marsh, seashore, lake, river (high productivity). Sexual dimorphism in feeding structures may be associated with intersexual niche differentiation (Shine 1989). Thus, according to the ecological flexibility hypothesis, rates of evolution of bill length dimorphism are expected to be higher in precocial species than semiprecocial species.

### **(c) Analyses**

We reconstructed the ancestral states of developmental mode (fig. 2) across the supertree phylogeny (Thomas *et al.* 2004) using maximum parsimony implemented in MacClade 4 (Maddison & Maddison 2001). More sophisticated methods (e.g. maximum likelihood) were not possible because of the prohibitively large amount of time it would take to reconstruct ancestral states for all 203 species for which we had data. To account for the uncertainty of developmental mode classification in eight species, we reconstructed ancestral states first with these species classed as precocial, and second with them classed as semiprecocial. The parsimony reconstruction was used to partition the phylogeny into the two matrices **V0** and **V1** as described in figure 1. In our analyses the matrix **V0** refers to the branch lengths shared amongst semiprecocial species, and **V1** refers to the branch lengths shared amongst precocial species.



**Figure 2.** Parsimony reconstruction showing the family and sub-family distribution of developmental mode in shorebirds. Black branches indicate precocial taxa, white branches indicate semiprecocial taxa.

Thomas *et al.* (2004) provide branch lengths in units of time but cautioned that they were crude estimates. We therefore performed all analyses with both the calibrated and equal branch lengths. In total, four sets of analyses were carried out on each trait: analysis *A* used the phylogeny with calibrated branch lengths with equivocal taxa classed as precocial; analysis *B* used the phylogeny with equal branch lengths with equivocal taxa classed as precocial; analysis *C* used the phylogeny with calibrated branch lengths with equivocal taxa classed as semiprecocial; and analysis *D* used the phylogeny with equal branch lengths with equivocal taxa classed as semiprecocial. The maximum likelihood value of the four analyses for a given trait can be compared and the one with the highest maximum likelihood value has the most statistical support. We report only the results of the set with the highest maximised log-likelihood value (the best fit to the data) for each trait tested except where the different sets yielded qualitatively different results. All analyses, including estimation of  $\theta$ , were performed

with R1.9.1 using code written by RPF. Note that the highest value  $\theta$  reported using this code is five, although the maximum likelihood value could be higher.

In addition, we tested the adequacy of the maximum likelihood estimation of  $\theta$  by randomisation under a Brownian motion model where  $\theta = 1$ . If the method performs adequately, the maximum likelihood estimate of  $\theta$  should not differ significantly from 1. We tested ordinal dummy data with three, five, and eight states (to match the range of ordinal data in our dataset) in addition to continuous dummy data. We found that for continuous data, the maximum likelihood value of  $\theta$  did not differ significantly from the expected value of one and that confidence intervals did not differ from those expected according to a  $\chi^2$  distribution (results not shown). However, ordinal data frequently resulted in biased estimates of  $\theta$  significantly different from the null model under which the dummy data was generated. We generated a distribution of 1000 maximum likelihood estimates of  $\theta$  to estimate the sampling interval and found that the mean estimate of  $\theta$  may be biased for our phylogenies and data. The reason is that if all species within a clade have a similar trait state then the variance within the clade for an ordinal trait tends to be much lower than for a continuous trait. This effect becomes exaggerated when clades differ in species number (in our data 91 taxa are precocial and 104 are semiprecocial, with eight intermediate taxa). Consequently, the maximum likelihood estimates of  $\theta$  for ordinal traits must be interpreted cautiously. We therefore compared the value of  $\theta$  with the simulated sampling interval in all tests involving ordinal data (see table 1). The ordinal traits in our analyses were male and female social mating system (five states), male and female parental care (eight states), and male display type and breeding habitat productivity (three states). If the maximum likelihood value of  $\theta$  falls within the confidence limits of the simulated sampling interval then there is no difference in the Brownian variance of the two groups, even if the likelihood ratio test suggests otherwise.

**Table 1.** Simulated sampling intervals of  $\theta$  for three, five, and eight state ordinal variables. The sampling intervals were estimated from dummy data for  $\theta = 1$  (1000 replicates). See text for the definitions of each set.

Number of states	Set	Lower confidence interval	Upper confidence interval
3	<i>A</i>	0.464	1.388
	<i>B</i>	0.673	1.754
	<i>C</i>	0.356	1.232
	<i>D</i>	0.368	1.177
5	<i>A</i>	0.482	1.337
	<i>B</i>	0.702	1.648
	<i>C</i>	0.343	1.248
	<i>D</i>	0.364	1.341
8	<i>A</i>	0.461	1.435
	<i>B</i>	0.560	1.369
	<i>C</i>	0.349	1.351
	<i>D</i>	0.363	1.278

### 3. RESULTS

#### (a) *Sexual conflict*

Rates of phenotypic evolution were higher amongst precocial shorebirds than amongst semiprecocial shorebirds for social mating system (male: *B*;  $\theta > 5.000$ ,  $\chi^2 = 48.890$ ,  $p < 0.001$ ,  $n = 158$ ; female: *A*;  $\theta > 5.000$ ,  $\chi^2 = 121.922$ ,  $p = 0.000$ ,  $n = 158$ ; fig. 3), and parental care (male: *A*;  $\theta > 5.000$ ,  $\chi^2 = 143.063$ ,  $p = 0.000$ ,  $n = 188$ ; female: *C*;  $\theta > 5.000$ ,  $\chi^2 = 148.470$ ,  $p = 0.000$ ,  $n = 188$ ; fig.3).

SSD in body mass evolved faster in precocial than in semiprecocial taxa (*D*;  $\theta = 2.879$ ,  $\chi^2 = 16.942$ ,  $p < 0.001$ ,  $n = 160$ ; fig. 3). However, SSD in wing length was dependent on the classification of the eight species that were intermediate in developmental mode. When classed as precocial, rates of evolution were higher in precocial taxa (*B*;  $\theta = 2.930$ ,  $\chi^2 = 14.340$ ,  $p < 0.001$ ,  $n = 189$ ), whereas when classed as semiprecocial there was no significant difference between precocial and semiprecocial taxa (*D*;  $\theta = 1.354$ ,  $\chi^2$

= 0.934,  $p = 0.333$ ,  $n = 189$ ). Three of these intermediate taxa fall at the extremes of sexual size dimorphism in wing length (*Philomachus pugnax*, *Rostratula benghalensis*, and *Scolopax minor*). Sequential removal of these taxa failed to yield consistent results across all four analyses (results not shown).

The results of male display type were equivocal. Three analyses (*A*, *C* and *D*) revealed no significant difference in rates of evolution between precocial and semiprecocial taxa (e.g. *A*;  $\theta = 1.469$ ,  $\chi^2 = 2.282$ ,  $p = 0.131$ ,  $n = 142$ ). Set *B* indicated that rates were higher amongst precocial species ( $\theta = 1.938$ ,  $\chi^2 = 6.889$ ,  $p = 0.009$ ,  $n = 142$ ). The respective fits of the models are difficult to adequately separate (*A* log-likelihood = -146.044; *B* log-likelihood = -145.932).

#### **(b) Life-histories**

Both body mass (*A*;  $\theta = 0.491$ ,  $\chi^2 = 9.827$ ,  $p = 0.002$ ,  $n = 160$ ; fig. 3), and wing length (*D*;  $\theta = 0.286$ ,  $\chi^2 = 33.352$ ,  $p = 0.035$ ,  $n = 189$ ; fig. 3) have higher rates of evolution amongst semiprecocial species. The results of the clutch size analyses were dependent on branch lengths: with calibrated branch lengths, clutch size evolved faster across semiprecocial species (*A*;  $\theta = 0.372$ ,  $\chi^2 = 19.155$ ,  $p < 0.001$ ,  $n = 166$ ); with equal branch lengths there was no difference (*B*;  $\theta = 0.849$ ,  $\chi^2 = 0.504$ ,  $p = 0.478$ ,  $n = 166$ ). The fit of the equal branch lengths model was better than the calibrated branch length model (log-likelihood = -125.193 and -130.993 respectively). Finally, rates of evolution of adult mortality were significantly higher amongst precocial taxa than semiprecocial taxa (*B*;  $\theta < 5.000$ ,  $\chi^2 = 29.495$ ,  $p < 0.001$ ,  $n = 61$ ; fig. 3)

#### **(c) Ecology**

As predicted, breeding habitat (*C*;  $\theta = 2.744$ ,  $\chi^2 = 10.825$ ,  $p = 0.001$ ,  $n = 84$ ; fig. 3) and SSD in bill length (*B*;  $\theta = 2.581$ ,  $\chi^2 = 16.753$ ,  $p < 0.001$ ,  $n = 164$ ; fig. 3) have higher rates of evolution amongst precocial species. However, breeding latitude had higher rates of evolution among semiprecocial species when calibrated branch lengths are used (*A*;  $\theta = 0.350$ ,  $\chi^2 = 26.118$ ,  $p < 0.001$ ,  $n = 200$ ) but not with equal branch lengths (*B*;  $\theta = 0.872$ ,  $\chi^2 = 0.463$ ,  $p = 0.496$ ,  $n = 200$ ). Equal branch lengths provide a better model fit than calibrated branch lengths (log-likelihood = -849.672 and -869.391 respectively).

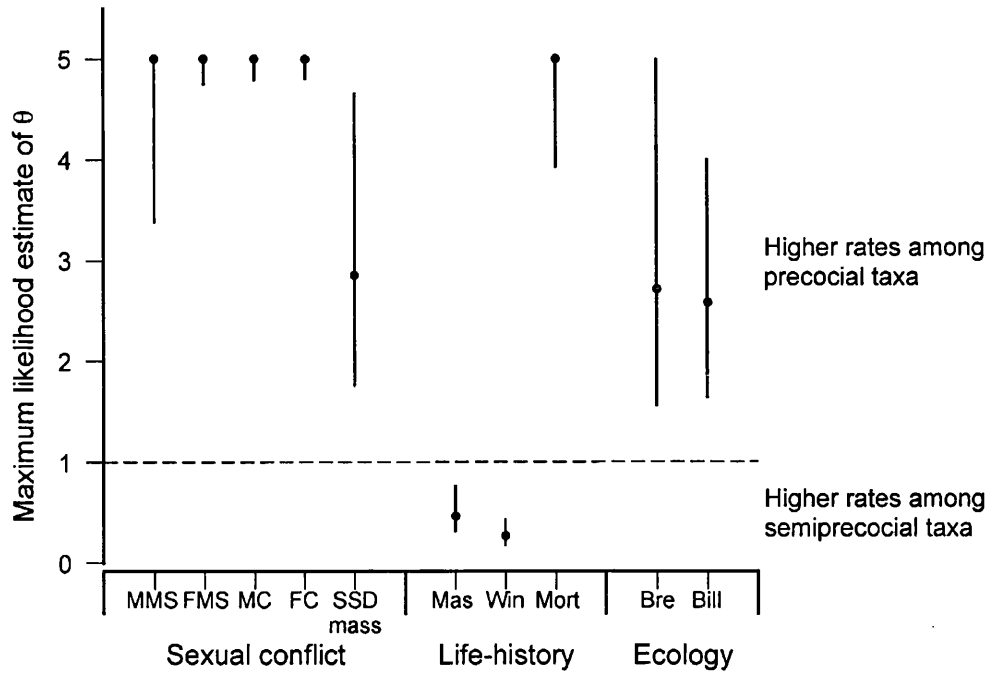


Figure 3. Maximum likelihood estimates of  $\theta$  with 95% confidence intervals for: male social mating system (MMS); female social mating system (FMS); male care (MC); female care (FC); sexual size dimorphism in body mass (SSD mass);  $\log_{10}$  body mass (Mas);  $\log_{10}$  wing length (Win); adult mortality (Mort); breeding habitat productivity (Bre); and, bill length dimorphism (Bill). Values of  $\theta > 1$  indicate rates of evolution are higher amongst precocial shorebirds; values of  $\theta < 1$  indicate rates of evolution are higher in semiprecocial taxa. Note that the upper confidence intervals are not included where the maximum likelihood estimate of  $\theta$  exceeds five.

#### 4. DISCUSSION

##### (a) *Developmental mode constrains phenotypic evolution*

Our analyses strongly suggest that developmental mode influences the diversification of traits associated with sexual conflict, life-histories, and ecology. After accounting for phylogeny we found that: (a) rates of evolution of mating and parental behaviour, and sexual size dimorphism are higher amongst species with precocial young than those with semiprecocial young; (b) life-history traits including body mass, and wing length have diverged more rapidly amongst semiprecocial taxa than precocial taxa, but adult



mortality shows the opposite trend; and (c) precocial species have diverged more rapidly in terms of habitat quality and bill length dimorphism.

We found that, as predicted, parental and mating behaviour diverged more rapidly amongst precocial taxa. This is highly consistent with Temrin & Tullberg's (1995) analyses across birds that showed that evolutionary transitions towards short pair bonds and polyandry were more frequent amongst precocial than altricial species. However, contrary to Temrin & Tullberg's (1995) results, we also found that polygyny is more frequent amongst precocial species. Our results also extend these conclusions to sexual selection in relation to sexual size dimorphism in body mass. The results for male display type were dependent on branch lengths of the phylogeny; however, it should be noted that male display is based on a crude scoring system. Detailed field observations of mating displays are absent for many species and improved resolution of this trait is thus required to resolve the ambiguity of our results.

Although we found that rates of evolution of body size were higher in semiprecocial taxa, the remainder of the life-history traits tested (clutch size, adult mortality) did not follow this pattern. The maximum clutch size across all shorebirds is generally regarded as four, thus, in comparison to the range across all birds (e.g. clutch sizes of over 15 in some megapodes; Bennett & Owens 2002) the potential variation is very low. As a consequence, differences in the rate of evolution of clutch size are likely to be difficult to detect. Notably, adult mortality varies more amongst precocial species. Sexual selection is expected to limit the ability to adapt to environmental change (Andersson 1994) and this is likely to act on precocial species more than semiprecocial species. However, the results may not have any adaptive significance since mortality varies greatly between populations of the same species and it is possible that this, coupled with a small sample size is responsible for this unexpected result.

The thermal independence of precocial offspring is expected to promote ecological flexibility, however, our results were inconclusive. Breeding habitat quality and sexual size dimorphism in bill length evolved more rapidly amongst precocial species. This suggests that precocial taxa have invaded a wider range of niches than semiprecocial taxa. However, the results for breeding latitude were equivocal. Overall, rates of

evolution of breeding latitude were slightly lower amongst precocial taxa, although this was non-significant in the best fitting model.

Taken together, our results consistently show that developmental mode has influenced the rates of phenotypic evolution of a range of traits. Whilst we cannot rule out alternative explanations for the variation in sexual selection, life histories, and ecology, it is unlikely that our results are linked to other aspects of ecological or life-history. Nest type has been inferred as an important constraint across birds but all shorebirds were classed as ground nesters in this analysis (Bennett & Owens 2002). Moreover, no relationship was found between clutch size and social mating system or parental care in shorebirds (Liker *et al.* 2001).

Recent studies suggest that most of the variation in life-history traits amongst birds was found at the family level or above (Owens & Bennett 1995). Furthermore, nesting site was identified as a possible constraint on life-histories (Bennett & Owens 2002) and it has been proposed that the evolution of avian life-histories and mating systems can be represented by a phylogenetic hierarchy (Owens & Bennett 1997; Bennett & Owens 2002). According to the hierarchical model some lineages are predisposed to certain traits or behaviours by features of their life-history that evolved deep within their evolutionary history, but ecological facilitation and social interactions determine the actual expression of these traits. Our results suggest that the potential expression of many phenotypic traits is predisposed by the developmental mode of the young. However, this implies that changes in developmental mode have *caused* changes in the rate of phenotypic evolution of other traits. Whilst this is highly plausible, it is not possible to separate cause and effect using this method. However, Thomas & Székely (submitted; see Chapter 5) found that evolutionary transitions from biparental care towards uniparental care occurred after changes from semiprecocial young to precocial young, with the implication that parental care is constrained by the developmental mode of the young. Nonetheless, it would be unwise to extend this conclusion to the full suite of traits investigated here without further testing.

#### **(b) Methodological issues**

Generalised least squares provides a highly flexible framework for the study of phylogenetic problems (e.g. Martins & Hansen 1997; Pagel 1997, 1999) and can be

applied to such problems as tests for correlated evolution between sets of characters, comparison of random walk and directional models of trait evolution, and the tempo and mode of trait evolution (Pagel 1997, 1999). Our new method extends this list to include comparison of rates of evolution between groups of taxa and overcomes several limitations of previous methods designed to address this problem. For instance, Garland (1992) proposed a method for comparing rates of phenotypic evolution in continuous traits among clades based on Felsenstein's (1985) independent contrasts. The basic tenet of Garland's (1992) approach is that the absolute value of the standardised contrasts represents the minimum amount of character change between one common ancestor and its two daughter taxa (assuming a bifurcating tree) under a constant-variance model of character evolution (Brownian motion). Differences in the mean rate of evolution between clades are then compared using standard statistical procedures ( $t$ -tests, Mann-Whitney  $U$ ). However, independent contrasts are calculated with respect to a common mean (i.e. the ancestral trait value at the root of the tree). By calculating a single set of contrasts, Garland's (1992) method assumes that the mean trait values of the two groups being compared are equal. If this assumption is not met, then any difference in contrasts between the groups under examination may be due to differences in their respective common means, rather than differences in their Brownian variance (i.e. clades with large mean trait values have larger contrasts than clades with small means). Equally, groups with different means could cloud real variation between groups. This approach may therefore be satisfactory only if the two groups have similar mean trait values, as might be expected when comparing monophyletic sister clades. If this is not the case, or if we wish to compare groups defined by criteria other than strict phylogenetic monophyly, then an alternative approach is required. Our approach overcomes these limitations by explicitly accounting for differences in the mean trait value of the groups under comparison thus minimizing the potential for detection of spurious differences between groups or of clouding real differences in trait evolution. Furthermore, our method can be used to compare between groups that need not be monophyletic. However, a major limitation is that we cannot separate cause and effect: differences in the rate of evolution of sexual size dimorphism *could* have constrained the evolution of developmental mode, however unlikely we believe this to be. Such problems can be tackled in a phylogenetic context (e.g. Pagel 1994) and it may be beneficial to use alternative methods alongside our approach. Finally, in most phylogenetic comparative analyses, the phylogeny is not known without error

(Huelsenbeck *et al.* 2000). In our analyses, this may manifest itself first as error in the phylogeny itself, and second as error in the reconstruction of the binary trait, in this case developmental mode. However, this is not a limitation of the method *per se*. In principle, a range of phylogenetic hypotheses (e.g. from a Bayesian distribution of trees) and ancestral state reconstructions could be incorporated.

In summary, this study provides evidence that developmental mode constrains the evolution of phenotypic evolution in shorebirds and allies, most notably in mating and parental behaviour. Our results are consistent with a hierarchical view of life-history and mating system evolution in which some lineages are predisposed to certain traits or behaviours by features of their early evolutionary history and recent ecological facilitation and social interactions determine the current expression of these traits. Finally, we have developed and used a new methodology that can be used to identify traits that limit rates of evolution in other phenotypic traits.

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**Chapter 5.** *Evolutionary pathways in shorebird breeding systems: sexual conflict, parental care and chick development*

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*Submitted manuscript*



## **Abstract**

Sexual selection, mating opportunities and parental behaviour are interrelated, although the specific nature of these relationships is controversial. Two major hypotheses have been suggested. On the one hand, the Parental Investment Hypothesis states that the relative parental investment of the sexes drives the operation of sexual selection. Thus, the sex that invests less in offspring care competes more intensely and monopolizes access to mates. On the other hand, the Sexual Conflict Hypothesis proposes that sexual selection (the competition among both males and females for mates), mating opportunities and parental behaviour are interrelated and predicts a feedback loop between mating competition and parental care. Here we test both hypotheses using a comprehensive dataset of shorebirds, a maximum-likelihood statistical technique and a recent supertree of extant shorebirds and allies. Shorebirds are an excellent group for these analyses since they display unique variation in parental care and social mating system. First, we show that chick development constrains the evolution of both parental care and mate competition, since transitions towards more precocial offspring preceded transitions towards reduced parental care and social polygamy. Second, changes in care and mating competition respond to one another. Finally, we show that changes in male care and female care respond to one another, and that neither sex consistently drives this relationship. Taken together, our results are more consistent with the Sexual Conflict Hypothesis than the Parental Investment Hypothesis.

## 1. INTRODUCTION

Parental care is beneficial for the growth and survival of offspring in many animals, whereas it is costly for the parents since it demands time and energy, and may limit the opportunities for finding a new mate (Clutton-Brock 1991; Balshine *et al.* 2002). Furthermore, care is often costly for the parent through decreased survival and future reproductive success. Thus in many multiple-breeding animals there is a conflict between the parents over who should care for the offspring (post-zygotic sexual conflict; Parker *et al.* 2002), with each parent preferring the other to do the hard work of raising young (Trivers 1972; Westneat & Sargent 1996; Lessells 1999; Houston *et al.* 2005). The sexual conflict over care may be resolved if one parent shifts the full amount of care to its mate by deserting, or if both parents remain with their brood and cooperate to raise the young (Houston & Davies 1985; Parker *et al.* 2002; Chapman *et al.* 2003).

The trade-off between costs and benefits of care has important implications for the evolution of parental care and mating strategies (Reynolds 1996; Székely *et al.* 2000a). Here we focus on this trade-off and address three key questions. First, do the demands of the offspring influence the trade-off between care and desertion, and thus the diversity of breeding systems? We predict that in species in which the demands of the offspring are small, for instance by being precocial, then the duration of parental care will be reduced and one parent will have the option of deserting and remating (Székely *et al.* 1996). Hence in precocial species a range of parental care and mating strategies, including social polyandry, social polygyny and lekking, is predicted (Orians 1969; Temrin & Tullberg 1995; Bennett & Owens 2002). In contrast, species with offspring that fully depend on their parents, for instance, by being unable to feed themselves, are likely to have high costs in terms of offspring survival if one parent deserts. Thus, in these species biparental care and social monogamy are predicted.

Second, do changes in parental care influence social mating systems, or do changes in social mating system drive changes in parental care? Two major hypotheses have been suggested. Trivers' (1972) Parental Investment Hypothesis is based upon Bateman's experiments (1948) and suggests that the relative parental investment of the sexes drives the operation of sexual selection. This hypothesis has led to important advances in theories of mating systems (Emlen & Oring 1977; Davies 1991). The hypothesis infers

that parental investment is a measure of the parent's ability to produce offspring in the future, and predicts that the sex that invests less in offspring will compete more intensely for mates, and can monopolize members of the care-giving sex. Thus, if females provide care, polygyny is expected, whereas if males provide care, polyandry is expected. In contrast, the Sexual Conflict Hypothesis suggests that mating and parental behaviours have feedback relationships, and sexual selection (e.g. the competition among both males and females for mates), mating opportunities and parental behaviour are mutually interrelated (Alonzo & Warner 2000; Székely *et al.* 2000a). For instance, a parent that has higher mating opportunity may desert and seek out new mates. Recent theoretical models (Barta *et al.* 2000; McNamara *et al.* 2000, 2002), observations (Pilastro *et al.* 2001) and experiments (Balshine-Earn & Earn 1998) have shown that this can result in a feedback loop between parental care and social mating system where these caring behaviours influence, and are influenced by, mating opportunities. The Parental Investment Hypothesis thus predicts that we should see evolutionary transitions towards uniparental care before transitions towards polygamous mating systems; changes from monogamy to polygamy should be more likely to occur if care is uniparental rather than biparental. In contrast, the Sexual Conflict Hypothesis predicts that mating and parental behaviours were evolved in feedback loops; transitions away from biparental care and social monogamy should be equally likely to proceed towards polygamy as towards uniparental care.

Third, we investigate whether patterns of care are driven primarily by males or by females. Traditional models of mating system and parental care suggest that male mating behaviour is influenced by the distribution and behaviour of females (Emlen & Oring 1977; Davies 1991), whereas the Sexual Conflict Theory suggests a tug-of-war between sexes over mating opportunities and parental care (Chapman *et al.* 2003; Arnqvist & Rowe in press; Houston *et al.* 2005). Thus the latter theory predicts feedback relations between parental decisions of males and females.

Shorebirds and allies (Aves: Charadriiformes; 350 species) are an excellent model system to investigate these questions due to their unique diversity of breeding systems amongst birds. Parental care ranges from fully biparental to male- or female-only care in shorebirds (Pitelka *et al.* 1974; Erckmann 1983; Székely & Reynolds 1995; Reynolds & Székely 1997), whilst social mating systems include polygyny (including lekking),

monogamy, multi-clutching, and most notably, polyandry (Pitelka *et al.* 1974; Erckmann 1983). Moreover, there is a dichotomy in their developmental mode between precocial (chicks self-feed) and semiprecocial (chicks unable to self-feed) taxa (see Starck & Ricklefs 1998 for a discussion on alternative definitions of developmental mode). The majority of sandpipers (Scolopacida) and plovers (Charadriida) are precocial whilst the gulls and alcids (Laridae) along with sheathbills *Chionis*, oystercatchers *Haematopus*, and thick-knees *Burhinidae* are semiprecocial (Székely & Reynolds 1995). Some alcids are referred to as precocial in the literature (e.g. Starck & Ricklefs 1998; Gaston 2003) but they are unusual in that whilst most semiprecocial birds do not leave the nest, some alcid chicks (e.g. the murrelets *Synthliboramphus* sp.; Perrins 2003) leave the nest soon after hatching. Significantly, however, they are still dependent on the parents for food after leaving the nest. Taken together, the range of breeding behaviour, and the diversity in precociality makes shorebirds and their allies an ideal group for assessing the links between developmental mode, parental care, and social mating system.

Previous comparative studies of shorebird breeding systems were informative, although they have not addressed the relationships between developmental mode, parental care, and social mating system. For instance, sexual size dimorphism correlates with mating system and male display behaviour (Figueroa 1999; Székely *et al.* 2000b; Székely *et al.* 2004), although the role of chick development and parental care have not been investigated. Other studies have shown that polygamous shorebirds lay eggs later, or they lay smaller eggs, than monogamous shorebirds (Whitfield & Tomkovich 1996; Liker *et al.* 2001), and that body mass and biparental against uniparental care explains variation in aggressive nest defense behaviour (Larsen *et al.* 1996). However, these studies have failed to disentangle cause and effect (Bennett & Owens 2002).

Directional comparative methods are required to distinguish the effects of developmental mode, parental care, and mating opportunities. In this paper we test the influence of developmental mode on shorebird breeding systems, and investigate two major hypotheses: the Parental Investment and the Sexual Conflict Hypotheses. In addition we examine the roles of the sexes in driving the evolution of parental care strategies. Our analyses use a directional phylogenetic approach (Pagel 1994) that can

detect the order and direction of evolutionary change in three important traits: developmental mode, parental care, and social mating system.

## 2. MATERIALS AND METHODS

### (a) *Data and phylogeny*

We collected data from across the order Charadriiformes, although the number of taxa included in our analyses was limited by the biological information available. The phylogeny of shorebirds is still in a state of flux. However, recent advances in molecular phylogenetics of the group (Ericson *et al.* 2003; Paton *et al.* 2003; Thomas *et al.* 2004a) and new analyses of morphological data (van Tuinen *et al.* 2004) suggest that an equitable consensus has been reached. We used the recent supertree of shorebirds (Thomas *et al.* 2004b) that is highly congruent with the molecular studies listed above. To our knowledge this supertree is the only shorebird phylogeny that has both complete taxonomic coverage (350 species) and estimates of branch lengths (based on molecular and fossil data), so that it provides the maximum statistical power currently available for comparative analyses for this group. All data are shown in Chapter 5 Appendix 1. We analyzed seven binary variables that can be described in three main groups; developmental mode, parental care, and social mating system.

*Developmental mode:* Each species was classified as either semiprecocial or precocial on the basis of the feeding ability of recently hatched young: semiprecocial chicks are unable to feed themselves immediately after hatching and typically remain in this state at least until fledging, whereas precocial young are able to feed themselves immediately after hatching. In some species the chicks cannot feed themselves immediately after hatching but are able to do so within one to two weeks (i.e. before fledging; see Chapter 5 Appendix 1). To investigate the sensitivity of our results to scoring these species either precocial or semiprecocial, we carried out all analyses of developmental mode twice, first with all seven species classified as precocial, then with them classified as semiprecocial. The results were generally consistent, however there were some qualitative differences. We therefore report only those results based on the precocial

coding of these seven taxa in the text and provide the full results of the semiprecocial coding in Chapter 5 Appendix 2.

*Parental care:* We used three variables for parental care: overall parental care, male care, and female care. All scores are based on whether care is provided until fledging of the young or not. For overall parental care, each species was classified as having care by both parents until fledging or reduced care by either parent. For male care, each species was classified as having either care by the male until fledging, or reduced care by the male, regardless of the parental behaviour of the female. Similarly, for female care, each species was classified as having care until fledging by the female or reduced care regardless of the parental behaviour of the male. We also use the term full care to describe care until fledging. Note that developmental mode and parental care were scored independently: developmental mode was purely based on the feeding ability of the young, whilst parental care was based on the duration of care including nest defense, brooding, and feeding.

*Social mating system:* We used three proxy measures of mate competition: overall mating system, male mating system, and female mating system. For overall mating system, each species was classified as either socially monogamous or socially polygamous (either polygynous or polyandrous). Male mating system was classified as male monogamy or male polygamy, regardless of the females' social mating system. Finally, female mating system was classified as either female monogamy or female polygamy, regardless of the males' social mating system. Note that in socially monogamous species the frequency of extra pair paternity is low (0-8% of broods; Griffith *et al.* 2002,  $n = 14$  species; Blomqvist *et al.* 2002,  $n = 3$  species). Note also that parental care and social mating systems were scored independently; parental care was purely based upon the duration of care, whereas mating system was based on social pair bond.

#### **(b) Phylogenetic Analyses**

We used Pagel's (1994) discrete variables method as implemented by the program DISCRETE (available from <http://www.ams.rdg.ac.uk/zoology/pagel/mppubs.html>). The method uses a continuous time Markov model, and allows tests of correlated

evolution as well as the order and direction of evolution for binary traits (Pagel 1994, 1997, 1999). Previous applications of this technique investigated the origins of sexual size dimorphism in ungulates (Pérez-Barbería *et al.* 2002), and the evolution of mate and site fidelity in Ciconiiforms (Cézilly *et al.* 2000). Models of evolution are fitted to the data and phylogeny using maximum likelihood, and the fit is described by the log-likelihood of the model. Correlated evolution is detected by comparing a model where two traits are allowed to evolve independently with one in which they are set to co-evolve. The independent model has four rate parameters; forward transitions from 0 to 1 and backward transitions from 1 to 0 for each of the two traits. In contrast, the dependent model has eight parameters in which the traits are allowed to coevolve. The eight possible transition rates of both characters are estimated simultaneously as combinations of single-state transitions.

The log-likelihoods of the two models are compared using the likelihood ratio statistic ( $LR = -2\log_e[L(I)/L(C)]$  where  $L(I)$  is the log-likelihood of the model of independent evolution, and  $L(C)$  is the log-likelihood of the model of dependent evolution). The  $LR$  statistic is generally expected to be distributed as a chi-squared variate with degrees of freedom equal to the difference in the number of parameters between the two models (Pagel 1994). The independent and dependent models differ by four parameters, but Pagel (1994) notes that the test of correlated evolution may not always follow the asymptotic chi-square distribution. However, simulations show that the appropriate degrees of freedom for the test of correlated evolution ranges from one to four (Pagel 1997). We therefore use four degrees of freedom since this is a conservative measure. The eight possible transitions, from state  $i$  to state  $j$  in the dependent model are characterized by the parameter  $q_{ij}$ . Pagel (1994) describes several tests in which one of the eight parameters is excluded *a priori* by forcing the relevant  $q_{ij}$  parameter to zero or to equal the maximum likelihood value of another rate parameter. This can be compared to the full dependent model, and the  $LR$  statistic for these tests is distributed as a chi-squared variate with one degree of freedom. We use the method of fixing a rate parameter to zero as this is a statistically more powerful test (see Cézilly *et al.* 2000 for an example of this approach).

We used DISCRETE to ask whether transitions away from a given ancestral state are more likely to proceed in one or the other direction. We also assess whether changes in

one trait are contingent on the state of the other trait. The ancestral states used in our analyses are semiprecocial offspring, biparental care until fledging, and social monogamy. We base these choices on previous studies (Székely & Reynolds 1995; Borowik & McLennan 1999; Reynolds *et al.* 2002) and on outgroup comparison with the Falconidae (the group with which the Charadriiformes share the closest common ancestor according to the phylogenetic hypothesis of Sibley & Ahlquist 1990). These ancestral states are consistent with maximum likelihood estimates of ancestral states made using DISCRETE (Pagel 1994). Since DISCRETE only accepts fully bifurcating trees, where we had data for more than two species in a single polytomy, we randomly selected two taxa. Specifically we asked: (1) did changes in developmental mode proceed before changes in parental care and social mating system; and (2) were changes in parental care or social mating dependent on the state of developmental mode? In addition, we tested all eight parameters in the dependent models of parental care and social mating system, and male and female care, to evaluate the predictions of both the Parental Investment and the Sexual Conflict hypotheses. If the data and phylogeny is consistent with the Parental Investment Hypothesis, then transitions from the ancestral state of biparental care and social monogamy should proceed first towards reduced parental care and social monogamy, and second from this state towards reduced parental care and social polygamy; no further transitions would be expected. In contrast, the Sexual Conflict Hypothesis predicts that changes away from biparental care and social monogamy are equally likely to proceed towards social polygamy first as they are towards reduced parental care. In addition, transitions back to biparental care or social monogamy from reduced care and social polygamy should occur. Such transitions could occur via either a switch back to biparental care or social monogamy. The likelihood surface for some parameters may be flat and the analysis may get stuck on local optima. As a result, sub-optimal maximum likelihood values were occasionally returned. We therefore repeated all analyses several times to ensure that we found the global maximum likelihood for each model.



### 3. RESULTS

#### *(a) Developmental mode and parental care*

Developmental mode correlated with overall parental care ( $\chi^2 = 15.491$ ,  $df = 4$ ,  $p = 0.004$ ; fig. 1a) and with female-only care ( $\chi^2 = 13.016$ ,  $df = 4$ ,  $p = 0.011$ ; fig. 1c), as predicted. No correlation was found between male care and developmental mode ( $\chi^2 = 5.217$ ,  $df = 4$ ,  $p = 0.266$ ; fig. 1b). The overall and female care results suggest that there was a two-step process from the ancestral state of semiprecocial young and full care to precocial young and reduced care.

*Step 1:* Changes in developmental mode preceded changes in parental care (fig. 1a,c). The transition from semiprecocial young with biparental care to precocial young with biparental care differs significantly from zero ( $\chi^2 = 8.428$ ,  $df = 1$ ,  $p = 0.004$ ; fig. 1a), whereas the transition from semiprecocial young with biparental care to semiprecocial young with reduced parental care does not differ significantly from zero ( $\chi^2 = 0.000$ ,  $df = 1$ ,  $p = 1.000$ ; fig. 1a). Transitions in female care are consistent with this pattern. The transition from semiprecocial young with full female care to precocial young with full female care differs significantly from zero ( $\chi^2 = 6.493$ ,  $df = 1$ ,  $p = 0.011$ ; fig. 1c), whereas the transition from semiprecocial young with full female care to semiprecocial young with reduced female care does not differ significantly from zero ( $\chi^2 = 0.000$ ,  $df = 1$ ,  $p = 1.000$ ; fig. 1c).

*Step 2:* Reductions in care are more likely to occur in species that have precocial young rather than semiprecocial young (fig. 1a,c). Transitions from precocial young with biparental care to precocial young with reduced care are marginally significantly different from zero ( $\chi^2 = 3.301$ ,  $df = 1$ ,  $p = 0.069$ ; fig. 1a), whereas transitions from semiprecocial young with biparental care to semiprecocial young with reduced care do not differ from zero (see above). Similarly, transitions from precocial young with full female care to precocial young with reduced female care differ significantly from zero ( $\chi^2 = 5.276$ ,  $df = 1$ ,  $p = 0.022$ ; fig. 1c), whereas transitions from semiprecocial young with full female care to semiprecocial young with reduced female care do not (see above).

Our results thus suggest that developmental mode first changed from semiprecocial to precocial and then one parent, most likely the female, reduced care over evolutionary time (fig. 1a,c). Note that the results for the semiprecocial coding were less strong (see Chapter 5 Appendix 2) but did not contradict the results of the precocial coding. None of our analyses suggested that parental care changed before developmental mode, or that the state of developmental mode was dependent on the state of parental care.

**(b) *Developmental mode and social mating system***

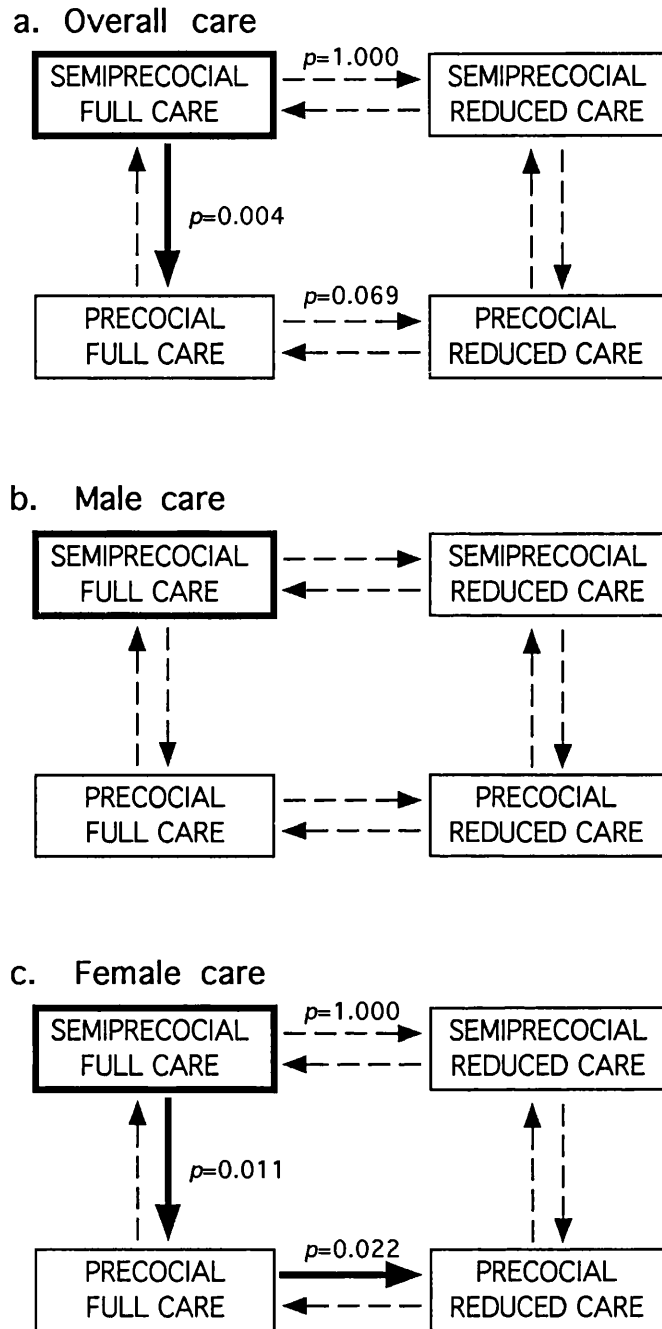
There is no relationship between developmental mode and social mating system ( $\chi^2 = 4.257$ ,  $df = 4$ ,  $p = 0.3723$ ; fig. 2a), and between developmental mode and mating system in males ( $\chi^2 = 2.717$ ,  $df = 4$ ,  $p = 0.6062$ ; fig. 2b). Developmental mode correlates with social mating system in females ( $\chi^2 = 10.634$ ,  $df = 4$ ,  $p = 0.031$ ; fig. 2c). As with parental care, there appears to be a two-step process in females linking the ancestral state of semiprecocial young and social monogamy to the derived precocial and social polygamy condition (fig. 2).

*Step 1:* Changes in developmental mode preceded changes in social mating system of females (fig. 2c). The transition from semiprecocial young with female monogamy to precocial young with female monogamy differs significantly from zero ( $\chi^2 = 10.579$ ,  $df = 1$ ,  $p = 0.001$ ; fig. 2c), but transitions from semiprecocial young with female monogamy to semiprecocial young with female polygamy do not ( $\chi^2 = 1.000$ ,  $df = 1$ ,  $p = 1.000$ ; fig. 2c).

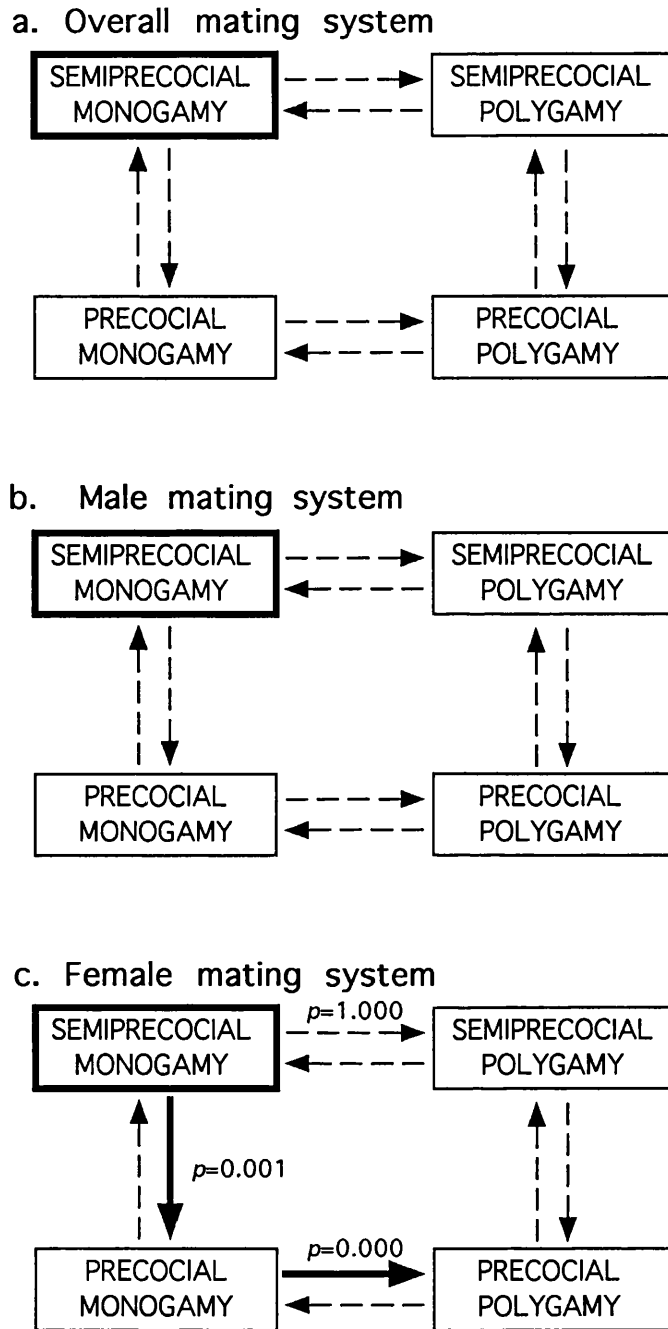
*Step 2:* Changes in female mating system towards social polygamy are more likely to occur in species that have precocial rather than semiprecocial young (fig. 2). Transitions from precocial young with female monogamy to precocial young with female polygamy differ significantly from zero ( $\chi^2 = 22.642$ ,  $df = 1$ ,  $p = 0.000$ ; fig. 2c), whereas transitions from semiprecocial young with female monogamy to semiprecocial young with female polygamy do not (see above).

As with parental care, the results for the semiprecocial coding were less strong (see Chapter 5 Appendix 2) but did not contradict the results of the precocial coding. None of our analyses suggested that social mating system changed before developmental

mode, or that the state of developmental mode was dependent on the state of social mating system.



**Figure 1.** Directional evolution of developmental mode and parental care in shorebirds and allies: (a) overall care, (b) male care, and (c) female care. Thick solid arrows show statistically significant pathways ( $p < 0.05$ ), all other pathways are marked with dashed arrows. Significance values are given for all tested pathways. Note that where the overall correlation was not statistically significant, tests of individual pathways were not performed. Highlighted boxes are the assumed ancestral states.



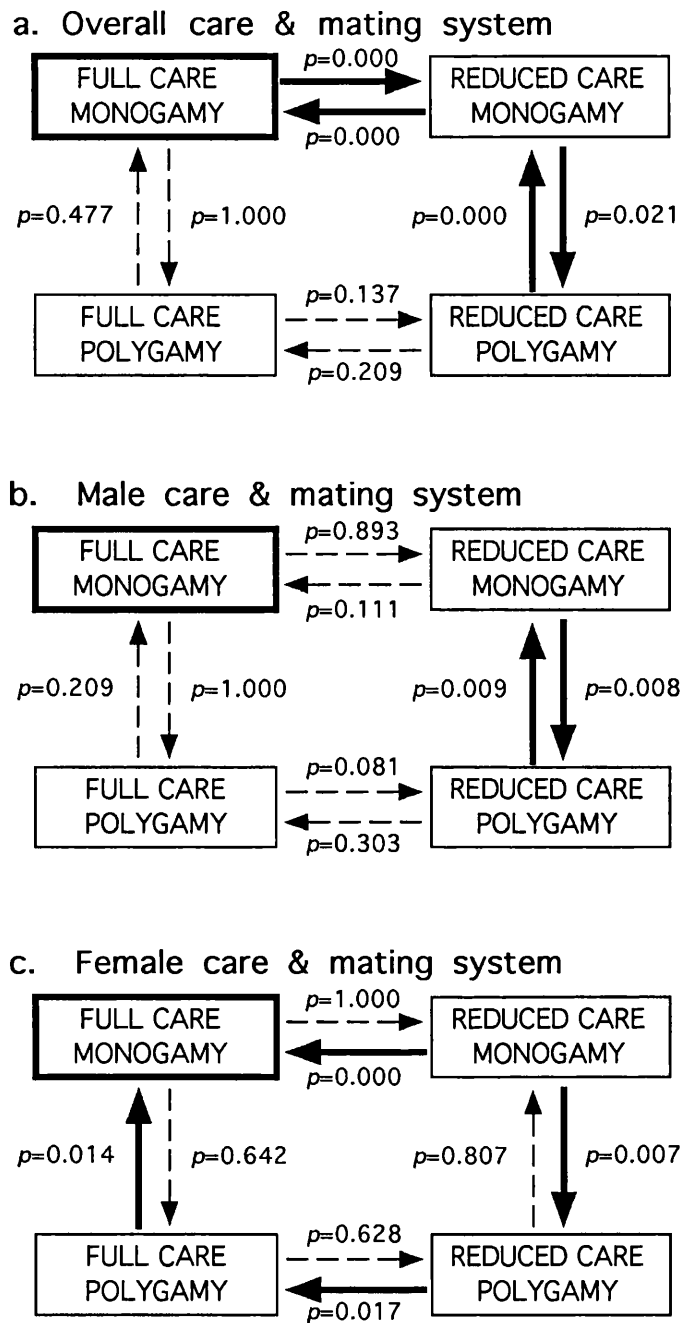
**Figure 2.** Directional evolution of developmental mode and social mating system in shorebirds and allies: (a) overall mating system, (b) male mating system, and (c) female mating system. Thick solid arrows show statistically significant pathways ( $p < 0.05$ ), all other pathways are marked with dashed arrows. Significance values are given for all tested pathways. Note that where the overall correlation was not statistically significant, tests of individual pathways were not performed. Highlighted boxes are the assumed ancestral states.

**(c) Parental care and social mating system**

Overall parental care and social mating systems were highly correlated ( $\chi^2 = 23.571$ ,  $df = 4$ ,  $p = 0.000$ ; fig. 3a). The relationships between male care and male mating system ( $\chi^2 = 42.995$ ,  $df = 4$ ,  $p = 0.000$ ; fig. 3b), and female care and female mating system ( $\chi^2 = 29.275$ ,  $df = 4$ ,  $p = 0.000$ ; fig. 3c) are also highly correlated.

*Overall parental care and social mating system (fig. 3a):* Transitions from biparental care with social monogamy to reduced care with social monogamy differ significantly from zero ( $\chi^2 = 14.700$ ,  $df = 1$ ,  $p = 0.000$ ), whereas transitions from biparental care with social monogamy to full parental care with social polygamy do not ( $\chi^2 = 0.000$ ,  $df = 1$ ,  $p = 1.000$ ). However, the reverse transition back from reduced care with social monogamy to full care with social monogamy is also significant ( $\chi^2 = 15.811$ ,  $df = 1$ ,  $p = 0.000$ ). The transition from reduced care with social monogamy to reduced care with social polygamy is significant ( $\chi^2 = 5.340$ ,  $df = 1$ ,  $p = 0.021$ ). The only other significant pathway detected was the transition from reduced care with social polygamy back to reduced care with social monogamy ( $\chi^2 = 14.832$ ,  $df = 1$ ,  $p = 0.000$ ). The feedback relationships between care and social mating systems are therefore more consistent with the Sexual Conflict Hypothesis than the Parental Investment Hypothesis.

*Male care and male mating system (fig. 3b):* We found no significant transitions away from the ancestral state for care and mating system of males. Transitions from full care with monogamy to reduced care with monogamy do not differ significantly from zero ( $\chi^2 = 0.018$ ,  $df = 1$ ,  $p = 0.893$ ). This is also true of transitions from full care with monogamy to full care with polygamy ( $\chi^2 = 0.000$ ,  $df = 1$ ,  $p = 1.000$ ). However, the transitions from reduced care with monogamy to reduced care with polygamy differ from zero ( $\chi^2 = 7.143$ ,  $df = 1$ ,  $p = 0.008$ ), and the transitions from reduced care with polygamy back to reduced care with monogamy also differ from zero ( $\chi^2 = 6.853$ ,  $df = 1$ ,  $p = 0.009$ ).



**Figure 3.** Directional evolution of parental care and social mating system in shorebirds and allies: (a) overall care and overall mating system, (b) male care and male mating system, and (c) female care and female mating system. Thick solid arrows show statistically significant pathways ( $p < 0.05$ ), all other pathways are marked with dashed arrows. All pathways were tested. Highlighted boxes are the assumed ancestral states.

*Female care and female mating system (fig. 3c):* Our results reveal a feedback loop between female care and female mating system that is consistent with the Sexual Conflict Hypothesis. Transitions from full care with monogamy to reduced care with monogamy do not differ significantly from zero ( $\chi^2 = 0.000$ ,  $df = 1$ ,  $p = 1.000$ ). This is also true of transitions from full care with monogamy to full care with polygamy ( $\chi^2 = 0.216$ ,  $df = 1$ ,  $p = 0.642$ ). This suggests that changes away from full care and monogamy in females are rare. However, from a state of reduced care and monogamy two pathways are strongly supported: transitions back to full care with monogamy ( $\chi^2 = 17.265$ ,  $df = 1$ ,  $p = 0.000$ ), and transitions to reduced care with polygamy ( $\chi^2 = 7.329$ ,  $df = 1$ ,  $p = 0.007$ ). The latter transition demonstrates a possible route to female desertion, polyandry and sex role reversal. The feedback loop is completed in two stages. First, the transition from reduced care with polygamy to full care with polygamy ( $\chi^2 = 5.674$ ,  $df = 1$ ,  $p = 0.017$ ), and then transitions from full care with polygamy to full care with monogamy ( $\chi^2 = 6.010$ ,  $df = 1$ ,  $p = 0.014$ ).

#### **(d) Male care and female care**

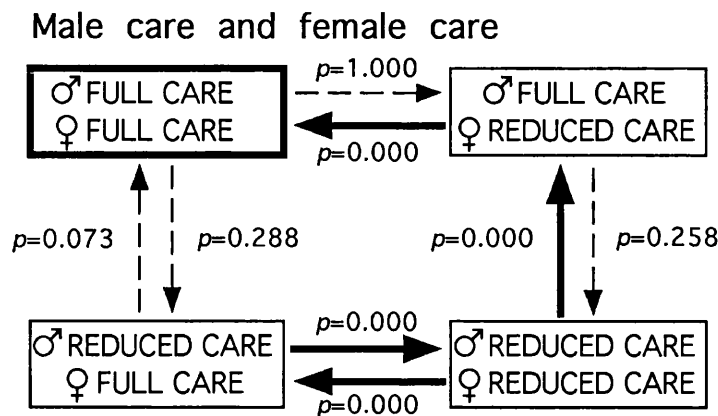
Taken together, our results so far are indicative of a female driven pattern of breeding system evolution consistent with the Sexual Conflict Hypothesis. We tested this further by considering the transitions in male care with female care (fig. 4).

Transitions in male care and female care were highly correlated ( $\chi^2 = 28.278$ ,  $df = 4$ ,  $p = 0.000$ ), as predicted. These transitions do not indicate which sex reduced its care from the ancestral states, since transitions from full care by the male and female to neither full male care and reduced female care ( $\chi^2 = 0.000$ ,  $df = 1$ ,  $p = 1.000$ ), nor to reduced male care and full female care differ significantly from zero ( $\chi^2 = 1.13$ ,  $df = 1$ ,  $p = 0.288$ ). However, from a state of full male care and reduced female care a complex series of transitions occur. Transitions back to full male care and full female care differ significantly from zero ( $\chi^2 = 12.816$ ,  $df = 1$ ,  $p = 0.000$ ), but transitions to reduced care in both sexes do not ( $\chi^2 = 1.28$ ,  $df = 1$ ,  $p = 0.258$ ). Reversal of the latter transition, back to full male care and reduced female, care is significant ( $\chi^2 = 18.318$ ,  $df = 1$ ,  $p = 0.000$ ). Transitions from reduced care by both sexes to reduced male care and full female care ( $\chi^2 = 13.038$ ,  $df = 1$ ,  $p = 0.000$ ) and back to reduced male care and reduced female care are significant ( $\chi^2 = 14.000$ ,  $df = 1$ ,  $p = 0.000$ ). Finally, transitions from reduced male



care and full female care to full care by both sexes is marginally non-significant ( $\chi^2 = 3.210$ ,  $df = 1$ ,  $p = 0.073$ ).

Our results suggest that, over evolutionary time, changes in male and female care respond to one another consistent with the Sexual Conflict Hypothesis, and neither males nor females appear to consistently drive these changes.



**Figure 4.** Directional evolution of male care and female care in shorebirds and allies. Thick solid arrows show statistically significant pathways ( $p < 0.05$ ), all other pathways are marked with dashed arrows. All pathways were tested. Highlighted boxes are the assumed ancestral states.

#### 4. DISCUSSION

Our results are consistent with the hypothesis that in more precocial species there will be a range of parental care and social mating strategies, whereas more altricial species will be restricted to biparental care and social monogamy (Orians 1969; Temrin & Tullberg 1995; Bennett & Owens 2002). We showed that developmental mode switched from semiprecocial to precocial before parental care reduced. This pattern holds for overall and female parental care. However, we found no relationship between developmental mode and male care. The predisposition of semiprecocial shorebirds to full biparental care suggests the feeding ability of recently hatched chicks is an important constraint in determining the possible outcomes of sexual conflict over care. Once this constraint is relaxed, for example, in precocial taxa, the outcomes of sexual conflict diversify. Our

results are thus consistent with the idea that certain traits predispose some lineages to a particular range of breeding systems, whereas ecological facilitation determines the observed phenotypic outcome (Owens & Bennett 1995, 1997; Bennett & Owens 2002). An alternative, though not mutually exclusive, explanation for the patterns of parental care and developmental mode is that of parent-offspring conflict (Trivers 1974). Trivers (1974) proposed that the parents respond to the demands of the young, whereas Alexander (1974) and Burley & Johnson (2002) argue that it is the offspring that respond to the behaviour of the parents. Whilst we do not provide direct tests for either hypothesis here, it is evident that our results are more closely aligned to Trivers (1974) explanation given that parental care appears to be a response to developmental mode rather than the reverse.

Our results also revealed that evolutionary changes in developmental mode proceeded before transitions in social mating system for female mating system. Note that in contrast to parental care and developmental mode, this relationship does not hold for overall mating system or male system. This suggests that developmental mode constrains the evolution of parental care but may not have a direct effect on social mating system.

We tested the Sexual Conflict and Parental Investment Hypotheses to explain the association between parental care and social mating system. Our results are highly consistent with the Sexual Conflict Hypothesis. In all analyses (overall, male and female), our results support a correlation between care and mating system (Lack 1968; Ligon 1999; Bennett & Owens 2002). Transitions in overall parental care away from biparental care proceed before transitions in social mating system in monogamy. The pattern of transitions for the female system is highly consistent with a sexual conflict feedback loop for the evolution of parental care and social mating system (fig. 3c). The feedback loop highlights the interrelationships between competition for mates, parental behaviour, and mating opportunities (Winkler & Wilkinson 1988; Alonzo & Warner 2000; Székely *et al.* 2000a). For example, a male reduces his duration of care and deserts since he has more opportunity to seek new mates; this leads to polygyny. However, competition between males for mates increases whereas competition between females decreases. Thus, the trade-off between the costs and benefits of desertion shift in favour of the male not deserting. This leads to transitions towards male monogamy.

The Parental Investment Hypothesis fails to account for the interactions between parental behaviour and mating opportunities and hence cannot explain the pattern of care and mating systems in shorebirds. Our phylogenetic results are consistent with the predictions of game theory models (Barta *et al.* 2000; McNamara *et al.* 2000, 2002), observations of parental care and mating system in the Dunnock *Prunella modularis* (Davies 1992) and experiments in the Kentish Plover *Charadrius alexandrinus* (Székely *et al.* 1999).

Our findings support the study of Reynolds & Székely (1997) in showing that male and female care are tightly correlated. Our results extend this work by showing that (1) there is a feedback loop between the parents over care, and (2) both sexes play an equally prominent role in driving this relationship. Our results thus differ from recent studies of primates that show that females drive social evolution (Lindenfors *et al.* 2003; Silk *et al.* 2003), and contradict the prediction that polygyny and lekking are an evolutionary dead-end in shorebirds (van Rhijn 1990).

Studies of other vertebrate groups, in particular teleost fish, have also revealed that male and female care are closely linked (Gittleman 1981; Gross and Sargeant 1985; Reynolds *et al.* 2002). Consistent with models (Gross & Sargeant 1985) and empirical studies (Goodwin *et al.* 1998) of evolutionary changes of care in fish, biparental care in shorebirds arises from mostly male care. In contrast, we found no support for a pathway between biparental care and female care. Direct comparisons between shorebirds and other vertebrate groups (e.g., teleosts, anurans) are confounded by the lack of any shorebird taxa in which neither parent provides care. For instance, male care arises from no care in fish, rather than from biparental care in shorebirds.

Taken together, our phylogenetic analyses show that the demands of the young constrain the possible outcomes of sexual conflict over parental care. Our results are consistent with the Sexual Conflict Hypothesis, whereas we do not find unambiguous support for the Parental Investment Hypothesis. Finally, we show that changes in male and female care are tightly correlated, and respond to one another. We encourage further phylogenetic tests of the hypotheses considered here across other taxonomic groups to elucidate the roles of offspring demand, and male and female behaviour in driving mating system and care patterns.

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## Appendix 1

Date used in the analyses and data sources. Developmental mode (0, semiprecocial; 1, precocial); overall parental care (0, neither parent deserts before fledging; 1, one or both parents desert before fledging); male care (0, male doesn't desert until fledging; 1, male deserts before fledging); female care (0, female doesn't desert until fledging; 1, female deserts before fledging); Overall mating system (0, monogamous; 1, polygamous); male mating system (0, male monogamy; 1, male polygamy); female mating system (0, female monogamy; 1, female polygamy). Developmental mode of species marked \* could be classified as precocial or semiprecocial, see text for details. In reference column, a = developmental mode; b = parental care; c = mating system; and # = no data).

Species	Developmental mode	Overall parental care	Male care	Female care	Overall mating system	Male mating system	Female mating system	References (a; b; c)
<i>Thinocorus orbignyianus</i>	1	1	1	0	0	0	0	2; 2; 2
<i>Thinocorus rumicivorus</i>	1	1	1	0	0	0	0	2; 2; 2
<i>Pedionomus torquatus</i>	1	1	0	1	1	0	1	3; 4; 5
<i>Scolopax rusticola</i> *	1	1	1	0	1	1	0	6; 7; 7
<i>Scolopax minor</i> *	1	1	1	0	1	1	0	9; 9; 9
<i>Gallinago hardwickii</i>		1	1	0	1	1	0	#; 10; 10
<i>Gallinago media</i>	1	1	1	0	1	1	0	6; 6; 6
<i>Gallinago gallinago</i> *	1	0	0	0				6; 11; #
<i>Lymnocyrtus minimus</i>		1	1	0	1	1	0	#; 6; 6
<i>Coenocorypha pusilla</i>	0	0	0	0	0	0	0	13; 12; 12
<i>Coenocorypha aucklandica</i>	0	0	0	0	0	0	0	13; 12; 13
<i>Limosa limosa</i>	1	0	0	0	0	0	0	6; 14; 6
<i>Limosa haemastica</i>	1	0	0	0	0	0	0	8; 15; 8
<i>Limosa lapponica</i>		0	0	0	0	0	0	#; 6; 6
<i>Limosa fedoa</i>	1	1	0	1	0	0	0	8; 16; 16
<i>Numenius phaeopus</i>	1	1	0	1	0	0	0	6; 17; 6
<i>Numenius tahitiensis</i>		1	0	1	0	0	0	#; 18; 18
<i>Numenius arquata</i>	1	1	0	1	0	0	0	6; 17; 6
<i>Numenius americanus</i>	1	1	0	1	0	0	0	39; 19; 19
<i>Tringa erythropus</i>	1	1	0	1				6; 20; #
<i>Tringa totanus</i>		1	0	1	0	0	0	#; 6; 6
<i>Tringa stagnatalis</i>		0	0	0	0	0	0	#; 6; 6
<i>Tringa nebularia</i>	1	1	0	1				6; 21; #
<i>Tringa melanoleuca</i>	1	0	0	0				22; 22; #
<i>Tringa ochropus</i>	1	1	0	1	0	0	0	6; 23; 6
<i>Tringa glareola</i>	1	1	0	1	0	0	0	6; 6; 6
<i>Tringa incana</i>	1	0	0	0				22; 22; #
<i>Catoptrophorus semipalmatus</i>	1	1	0	1	0	0	0	24 24; 24
<i>Arenaria interpres</i>	1	1	0	1	0	0	0	6; 25; 25
<i>Arenaria melanocephala</i>	1	0	0	0	0	0	0	39; 26; 26
<i>Limnodromus griseus</i>	1	1	0	1	0	0	0	22; 27; 8
<i>Limnodromus scolopaceus</i>	1	1	0	1	0	0	0	22; 28; 28
<i>Limnodromus semipalmatus</i>		1	0	1				#; 29; #
<i>Calidris alba</i>	1	1	0	1	1	0	1	6; 30; 30
<i>Calidris pusilla</i>		1	0	1	0	0	0	#; 31; 31
<i>Calidris mauri</i>	1	1	0	1	0	0	0	58; 32,33; 32

<i>Calidris minuta</i>		1	0	1	1	0	1	#; 6; 6
<i>Calidris ruficollis</i>		1	0	1	0	0	0	#; 34; 34
<i>Calidris temminckii</i>	1	1	0	1	1	0	1	6; 6,35; 35
<i>Calidris subminuta</i>		1	0	1	0	0	0	#; 36; 34
<i>Calidris minutilla</i>	1	1	0	1				22; 37; #
<i>Calidris fuscicollis</i>	1	1	1	0	1	1	0	8; 38; 38
<i>Calidris bairdii</i>	1	1	0	1				8; 34; #
<i>Calidris melanotos</i>	1	1	1	0	1	1	0	22; 40; 40
<i>Calidris acuminata</i>		1	1	0	1	1	0	#; 34; 34
<i>Calidris maritima</i>	1	1	0	1				6; 6; #
<i>Calidris ptilocnemis</i>	1	1	0	1	0	0	0	6; 34; 34
<i>Calidris alpina</i>	1	1	1	1	0	0	0	6; 41; 41
<i>Tryngites subruficollis</i>	1	1	1	0	1	1	0	22; 42; 42
<i>Limicola falcinellus</i>	1	1	0	1	0	0	0	6; 6; 6
<i>Philomachus pugnax*</i>	1	1	1	0	1	1	0	6; 6; 6
<i>Steganopus tricolor</i>	1	1	0	1	1	0	1	22; 43; 44
<i>Phalaropus lobatus</i>	1	1	0	1	1	0	1	6; 45; 6
<i>Phalaropus fulicaria</i>	1	1	0	1	1	0	1	6; 46; 6
<i>Rostratula benghalensis*</i>	1	1	0	1	1	0	1	8; 47; 6
<i>Rostratula semicollaris</i>		0	0	0	0	0	0	#; 1; 48
<i>Actophilornis africanus</i>	1	1	0	1	1	0	1	50; 49; 50
<i>Microparra capensis</i>	1	0	0	0	0	0	0	39; 51; 51
<i>Irediparra gallinacea</i>		1	0	1	1	0	1	#; 28; 28
<i>Hydrophasianus chirurgus</i>	1	1	0	1	1	0	1	87; 52; 53
<i>Metopidius indicus</i>		1	0	1	1	0	1	#; 54; 8
<i>Jacana spinosa</i>	1	1	0	1	1	0	1	8; 55; 55
<i>Jacana jacana</i>	1	1	0	1	1	0	1	22; 56; 56
<i>Chionis alba</i>	0	0	0	0	0	0	0	3; 57; 57
<i>Chionis minor</i>	0	0	0	0	0	0	0	3; 58; 58
<i>Pluvianellus socialis</i>	0	0	0	0	0	0	0	8; 59; 8
<i>Burhinus oedicephalus</i>	0	0	0	0	0	0	0	6; 60; 60
<i>Burhinus senegalensis</i>		0	0	0	0	0	0	#; 61; 50
<i>Burhinus capensis</i>	0	0	0	0	0	0	0	50; 50; 50
<i>Burhinus grallarius</i>	0	0	0	0	0	0	0	3; 62; 62
<i>Haematopus ostralegus</i>	0	0	0	0	0	0	0	5; 63; 63
<i>Haematopus moquini</i>	0	0	0	0				50; 64; #
<i>Haematopus finschi</i>		0	0	0	0	0	0	#; 3; 3
<i>Haematopus bachmani</i>		0	0	0	0	0	0	#; 65; 8
<i>Haematopus palliatus</i>	0	0	0	0	0	0	0	8; 66; 8
<i>Haematopus longirostris</i>	0	0	0	0	0	0	0	1; 67; 67
<i>Haematopus unicolor</i>		0	0	0	0	0	0	#; 68; 3
<i>Haematopus fuliginosus</i>	0	0	0	0	0	0	0	67; 67; 67
<i>Ibidorhyncha struthersii</i>	0	0	0	0	0	0	0	8; 69; 69
<i>Himantopus himantopus</i>	1	0	0	0	0	0	0	6; 6; 6
<i>Himantopus novaezelandiae</i>		0	0	0	0	0	0	#; 70; 70
<i>Himantopus mexicanus</i>	1	0	0	0				22; 71; #
<i>Cladorhynchus leucocephalus</i>		0	0	0				#; 3; #
<i>Recurvirostra avosetta</i>	1	0	0	0	0	0	0	6; 71; 71
<i>Recurvirostra americana</i>	1	0	0	0	0	0	0	72; 72; 72
<i>Recurvirostra novaehollandiae</i>		0	0	0	0	0	0	#; 3; 3
<i>Pluvialis apricaria</i>	1	1	0	1	0	0	0	6; 21; 6
<i>Pluvialis fulva</i>	1	1	0	1	0	0	0	22; 28; 28
<i>Pluvialis dominica</i>	1	0	0	0	0	0	0	73; 73; 73
<i>Pluvialis squatarola</i>	1	1	0	1	0	0	0	6; 6; 6

<i>Charadrius hiaticula</i>	1	1	0	1	0	0	0	6; 6; 6
<i>Charadrius semipalmatus</i>	1	1	0	1	0	0	0	22; 22; 22
<i>Charadrius dubius</i>	1	1	0	1	0	0	0	6; 74; 6
<i>Charadrius tricollaris</i>	1	0	0	0	0	0	0	50; 50; 50
<i>Charadrius wilsonia</i>	1	0	0	0	0	0	0	8; 75; 6
<i>Charadrius vociferus</i>	1	0	0	0	0	0	0	8; 76; 76
<i>Charadrius melodus</i>	1	0	0	0	0	0	0	22; 22; 22
<i>Charadrius bicinctus</i>	1	0	0	0				3; 3; #
<i>Charadrius mongolus</i>		1	0	1				#; 53; #
<i>Charadrius asiaticus</i>	1	0	0	0				6; 28; #
<i>Thinornis novaeseelandiae</i>		0	0	0	0	0	0	#; 3; 3
<i>Erythronyx cinctus</i>		0	0	0				#; 3; #
<i>Eudromias morinellus</i>	1	1	0	1				6; 77; #
<i>Anarhynchus frontalis</i>		0	0	0	0	0	0	#; 78; 78
<i>Peltohyas australis</i>		0	0	0	0	0	0	#; 3; 3
<i>Vanellus crassirostris</i>		0	0	0	0	0	0	#; 79; 50
<i>Vanellus miles</i>		0	0	0	0	0	0	#; 68; 68
<i>Vanellus armatus</i>	1	0	0	0	0	0	0	50; 80; 50
<i>Vanellus spinosus</i>	1	0	0	0	0	0	0	6; 6; 6
<i>Vanellus lugubris</i>	1	0	0	0				50; 81; #
<i>Vanellus gregarius</i>	1	0	0	0				6; 6; #
<i>Vanellus chilensis</i>	1	0	0	0	0	0	0	79; 79; 79
<i>Rhinoptilus africanus</i>	0	0	0	0	0	0	0	1; 82; 50
<i>Rhinoptilus cinctus</i>		0	0	0				#; 50; #
<i>Cursorius cursor</i>		0	0	0	0	0	0	#; 6; 6
<i>Cursorius coromandelicus*</i>	1	0	0	0	0	0	0	88; 6; 6
<i>Glareola pratincola</i>	0	0	0	0	0	0	0	1; 6; 6
<i>Glareola maldivarum</i>		0	0	0	0	0	0	#; 86; 86
<i>Glareola cinerea</i>	0	0	0	0				50; 50; #
<i>Stiltia isabella</i>		0	0	0	0	0	0	#; 13; 13
<i>Rynchops flavirostris</i>	0	0	0	0	0	0	0	50; 50; 50
<i>Rynchops niger</i>	0	0	0	0				83; 83; #
<i>Catharacta skua</i>	0	0	0	0	0	0	0	13; 13; 13
<i>Catharacta maccormickii</i>	0	0	0	0	0	0	0	13; 13; 13
<i>Stercorarius pomarinus</i>	0	0	0	0	0	0	0	6; 6; 6
<i>Stercorarius parasiticus</i>		0	0	0	0	0	0	#; 6; 6
<i>Larus hemprichii</i>	0	0	0	0				50; 50; #
<i>Larus canus</i>	0	0	0	0	0	0	0	6; 6; 6
<i>Larus delawarensis</i>	0	0	0	0	0	0	0	22; 22; 22
<i>Larus californicus</i>	0	0	0	0	0	0	0	22; 22; 22
<i>Larus marinus</i>		0	0	0				#; 6; #
<i>Larus dominicanus</i>	0	0	0	0	0	0	0	50; 13; 13
<i>Larus glaucescens</i>	0	0	0	0	0	0	0	22; 13; 13
<i>Larus occidentalis</i>	0	0	0	0	0	0	0	22; 22; 22
<i>Larus livens</i>	0	0	0	0	0	0	0	22; 22; 22
<i>Larus hyperboreus</i>	0	0	0	0	0	0	0	22; 6; 6
<i>Larus argentatus</i>		0	0	0	0	0	0	#; 6; 6
<i>Larus fuscus</i>	0	0	0	0				6; 6; #
<i>Larus ichthyaetus</i>	0	0	0	0				6; 6; #
<i>Larus cirrocephalus</i>	0	0	0	0	0	0	0	6; 6; 6
<i>Larus novaehollandiae</i>	0	0	0	0	0	0	0	13; 13; 13
<i>Larus bulleri</i>		0	0	0	0	0	0	#; 13; 13
<i>Larus ridibundus</i>	0	0	0	0	0	0	0	6; 6; 6
<i>Larus genei</i>	0	0	0	0	0	0	0	6; 6; 6

<i>Larus philadelphia</i>	0	0	0	0	0	0	0	22; 22; 22
<i>Larus melanocephalus</i>	0	0	0	0	0	0	0	6; 6; 6
<i>Larus atricilla</i>	0	0	0	0	0	0	0	22; 22; 22
<i>Larus pipixcan</i>	0	0	0	0	0	0	0	22; 22; 22
<i>Larus minutus</i>	0	0	0	0	0	0	0	6; 6; 6
<i>Pagophila eburnea</i>	0	0	0	0	0	0	0	22; 6; 6
<i>Xema sabini*</i>	1	0	0	0	0	0	0	6; 6; 6
<i>Rissa tridactyla</i>	0	0	0	0	0	0	0	6; 6; 6
<i>Rissa brevirostris</i>	0	0	0	0	0	0	0	22; 22; 22
<i>Sterna bergii</i>		0	0	0	0	0	0	#; 13; 13
<i>Sterna hirundo</i>	0	0	0	0				22; 84; 84
<i>Sterna paradisea</i>	0	0	0	0				84; 84; #
<i>Sterna dougallii</i>	0	0	0	0				84; 84; #
<i>Sterna striata</i>		0	0	0	0	0	0	#; 13; 13
<i>Sterna vittata</i>	0	0	0	0				13; 13; #
<i>Chlidonias hybridus</i>	0	0	0	0	0	0	0	85; 84; 84
<i>Chlidonias leucopterus</i>	0	0	0	0	0	0	0	84; 84; 84
<i>Chlidonias niger</i>	0	0	0	0	0	0	0	6; 6; 6
<i>Anous stolidus</i>	0	0	0	0				13; 13; #
<i>Anous minutus</i>	0	0	0	0	0	0	0	13; 13; 13
<i>Gygis alba</i>	0	0	0	0	0	0	0	13; 13; 13
<i>Alle alle</i>	0	0	0	0	0	0	0	84; 84; 84
<i>Uria aalge</i>	0	0	0	0	0	0	0	84; 84; 84
<i>Uria lomvia</i>	0	0	0	0	0	0	0	84; 84; 84
<i>Alca torda</i>	0	0	0	0	0	0	0	84; 84; 84
<i>Cephus grylle</i>	0	0	0	0	0	0	0	85; 85; 85
<i>Cephus columba</i>	0	0	0	0	0	0	0	28; 28; 28
<i>Brachyramphus marmoratus</i>	0	0	0	0	0	0	0	85; 85; 85
<i>Synthliboramphus hypoleucus</i>	0	0	0	0	0	0	0	85; 85; 85
<i>Synthliboramphus antiquus</i>	0	0	0	0	0	0	0	22; 22; 22
<i>Cyclorhynchus psittacula</i>	0	0	0	0	0	0	0	85; 85; 85
<i>Aethia cristatella</i>	0	0	0	0	0	0	0	85; 85; 85
<i>Aethia pusilla</i>	0	0	0	0	0	0	0	85; 85; 85
<i>Cerorhinca monocerata</i>	0	0	0	0	0	0	0	22; 22; 22
<i>Fratercula arctica</i>	0	0	0	0	0	0	0	84; 84; 84
<i>Fratercula cirrhata</i>	0	0	0	0				84; 84; #

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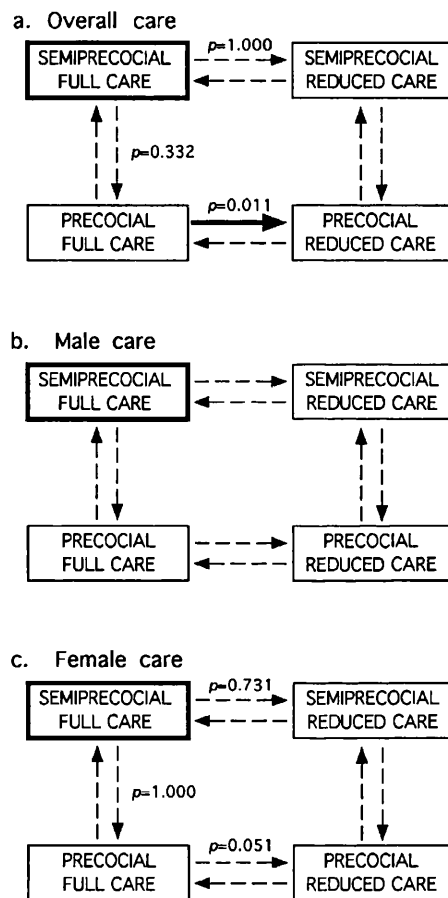
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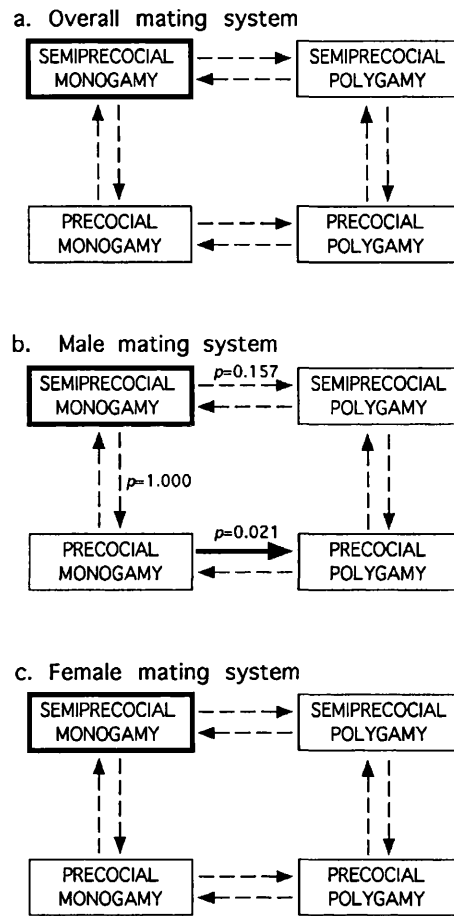
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## Appendix 2

The relationship between developmental mode and parental care/social mating system is less strong when intermediate taxa are coded as semiprecocial rather than precocial. Indeed, there was only a significant correlation between developmental mode and overall parental care, female care, and male mating system. We were unable to identify whether transitions in developmental mode occurred before transitions in parental care/social mating system or vice versa. Nonetheless, changes in parental care and social mating system were shown to be dependent on the state of developmental mode being precocial. None of the results from the semiprecocial coding analyses conflict with those from the precocial coding analyses (i.e. they don't suggest that parental care or social mating system changed before developmental mode, or that developmental mode is dependent on the state of either parental care or social mating system).



**Figure 5.** Directional evolution of developmental mode and parental care in shorebirds and allies: (a) overall care ( $\chi^2 = 15.795$ ,  $df = 4$ ,  $p = 0.003$ ), (b) male care ( $\chi^2 = 6.606$ ,  $df = 4$ ,  $p = 0.158$ ), and (c) female care ( $\chi^2 = 14.863$ ,  $df = 4$ ,  $p = 0.005$ ). Thick solid arrows show statistically significant pathways ( $p < 0.05$ ), all other pathways are marked with dashed arrows. Where the overall correlation was not statistically significant, tests of individual pathways were not performed. Highlighted boxes are the assumed ancestral states.



**Figure 6.** Directional evolution of developmental mode and social mating system in shorebirds and allies: (a) overall mating system ( $\chi^2 = 6.102$ ,  $df = 4$ ,  $p = 0.192$ ), (b) male mating system ( $\chi^2 = 11.191$ ,  $df = 4$ ,  $p = 0.025$ ), and (c) female mating system ( $\chi^2 = 5.926$ ,  $df = 4$ ,  $p = 0.205$ ). Thick solid arrows show statistically significant pathways ( $p < 0.05$ ), all other pathways are marked with dashed arrows. Where the overall correlation was not statistically significant, tests of individual pathways were not performed. Highlighted boxes are the assumed ancestral states.

**Chapter 6.** *Rates of diversification and correlates of species richness in shorebirds, gulls, and alcids*

Gavin H. Thomas

*Submitted manuscript*

## **Abstract**

Variation in species richness across the tree of life may be due to chance alone or the result of sexual selection, constraints on body size and life-history, ecology, and biogeography. I investigated patterns and correlates of species richness in shorebirds, gulls, and alcids (350 extant species). I show that speciation in shorebirds is not consistent with purely stochastic processes, and that there were at least three major changes in diversification rates across the shorebird tree. Sexual selection drives speciation in shorebirds but the mechanisms differ between lineages. In sandpipers and allies, acrobatic aerial display flights, indicating female mate choice, are associated with increased species richness. In contrast, more polyandrous mating systems, suggesting increased sexual selection on females, are associated with increased species richness amongst the plovers and allies. In the absence of notable sexual selection, increased fecundity is a marginal correlate of speciation in the gulls and allies.

## 1. INTRODUCTION

Why are some families species rich whilst others contain only a single species? There are two major challenges to understanding the disproportionate distribution of species across phylogenetic trees. First, does the distribution of species differ from some null model of cladogenesis (Purvis 1996)? Second, if purely stochastic processes cannot adequately explain patterns of species richness, what intrinsic biological or extrinsic environmental processes have driven some lineages to diversify more rapidly than others (Purvis 1996; Schluter 2000; Coyne & Orr 2004)?

The equal rates Markov model of cladogenesis (ERM; Chan & Moore 2002) assumes that every lineage has the same probability of speciating at any point in time (Yule 1924). Deviation from this model is taken as evidence that purely stochastic processes cannot explain patterns of species richness for a given tree (Purvis 1996). This model has been used to demonstrate that in many cases the tree of life is significantly more imbalanced than we would expect by chance alone. However, general explanations for differential rates of cladogenesis across phylogenies have proven elusive (Vogler & Ribera 2003). Recent comparative studies across a wide range of taxa have yielded equivocal results. For instance, small body-size was associated with more species rich clades in primates (Gittleman & Purvis 1998) whereas no relationship was found in carnivores (Gittleman & Purvis 1998), birds (Owens *et al.* 1999), Australian mammals (Cardillo *et al.* 2003), hoverflies (Katzourakis *et al.* 2001), Metazoa (Orme *et al.* 2002), or agamid lizards (Stuart-Fox & Owens 2003). Furthermore, amongst birds, some studies suggest that more intense sexual selection is associated with increased species richness (Owens *et al.* 1999; Mitra *et al.* 1996) whereas others found no relationship (Morrow *et al.* 2003). It is evident that the factors that promote speciation may differ between lineages. Multiple hypotheses have been suggested to explain variation in species in richness.

### **(a) *Sexual selection and sexual conflict***

Sexual selection by female mate choice may result in the coevolution of “attractiveness” traits in male and female choice of such traits if females that mate with more attractive males also have more attractive sons (Fisher 1930). Thus, secondary sexual characters



can evolve very rapidly in a positive feedback runaway process (Fisher 1930). According to the sexual selection hypothesis, rapid divergence in female mate choice is likely to result in the formation of reproductive barriers in isolated populations and thus promote sexual selection (Lande 1981; West-Eberhard 1983; Barraclough *et al.* 1998; Panhuis *et al.* 2001). Recently, sexual conflict has been identified as an important alternative driver for speciation that is closely related to the sexual selection hypothesis (Parker & Partridge 1998; Arnqvist *et al.* 2000; Gavrillets 2000; Gavrillets & Waxman 2002; Martin & Hosken 2003). Sexual conflict arises since males typically invest less in direct offspring care than females (Trivers 1972). Males are therefore limited by the number of females they can fertilise whereas females are limited by the number of eggs they can produce and the quality of their mates (Bateman 1948; Gage *et al.* 2002). The mating optima of males and females are clearly different and this can lead to the evolution of sexually selected characteristics along antagonistic, rather than mutualistic, trajectories (Parker 1979; Chapman *et al.* 2003; Pizzari & Snook 2003). Adaptations that benefit one sex at the expense of the other should drive counter-adaptations to avoid exploitation by the other sex (Trivers 1972). Consequently, sexual conflict may drive the rapid divergence of sexual characteristics under selection in the form of a chase-away arms race (Pizzari & Snook 2003). Sexual conflict is expected to result in different rates of coevolution of males and females in isolated populations (Chapman *et al.* 2003) and thus promote speciation (Arnqvist *et al.* 2000).

**(b) *Life-history***

Species with short generation times and high reproductive rates are expected to evolve faster than species with long generation times and low reproductive rates. Therefore, species with fast life-histories should have higher speciation rates and lower extinction rates (Marzluff & Dial 1991; Owens *et al.* 1999; Katzourakis *et al.* 2001; Stuart-Fox & Owens 2003).

**(c) *Niche availability***

The niche availability hypothesis states that taxa that can invade new niches are more likely to be species rich than taxa with limited niche availability. Several factors may influence niche availability. First, habitat or food generalists are more likely to be able to colonize new regions than species with very specific habitat or food demands (Simpson 1953; Mayr 1963; Rosenzweig 1995). Second, it has also been suggested that

species richness increases at lower latitudes (Rosenzweig 1995). Third, small-bodied species require fewer resources than large-bodied species so are expected to be able to exploit a wider range of niches (Hutchinson & MacArthur 1969; Van Valen 1973; Stanley 1973; May 1986). Finally, species with large total range sizes are more likely to be able to invade new niches (Rosenzweig 1995; Gaston & Blackburn 1997). However, speciation events should be associated with the splitting of ranges through geographic isolation. Thus, species with widespread but fragmented ranges (small mean population range sizes) are expected to be more speciose (Gaston & Blackburn 1997).

#### **(d) *Shorebirds, gulls, and alcids***

The aim of this study is to identify correlates of species richness in shorebirds (Aves: Charadriiformes, 350 species; Monroe & Sibley 1993). Shorebirds are ideally suited to studies of species richness for several reasons. First, there is a complete supertree phylogeny for the group (Thomas *et al.* 2004), so the distribution of species across the tree can be tested without the undesirable recourse to an arbitrary choice of taxonomy (Moore *et al.* 2004). Second, shorebirds display the widest range of mating and parental behaviour of any avian order (Reynolds & Székely 1997). Thus, we expect to see a gradient in the intensity of both sexual selection and sexual conflict acting across the group (Thomas & Székely submitted, Chapter 5). In addition, shorebirds display a wide range of body sizes (from the 20 gram least sandpiper *Calidris minutilla* to the almost two kilogram great skua *Catharacta skua*) and life-histories (including common and thick-billed murres that breed for over 20 years; Gaston & Jones 1998). Finally, shorebirds occupy diverse ecological and biogeographic regions including species breeding in the high Arctic (numerous sandpipers), Antarctic (sheathbills), tropics (jacanas, painted-snipes), temperate wetlands (oystercatchers and stilts), and arid regions (pratincoles and coursers).

Here, I employ an ERM model of cladogenesis to test for variation in diversification rates in shorebirds. I then use two recently developed indices to identify nodes in the tree associated with major changes in the rate of diversification. Finally, I test each of the hypotheses outlined above to explain variation in species richness amongst shorebirds.

## 2. MATERIALS AND METHODS

### (a) *Phylogeny*

I used Thomas *et al.*'s (2004) supertree of shorebirds in all analyses. The supertree includes all 350 species of shorebirds and is the only complete phylogeny for the extant members of the order. Alternative phylogenetic hypotheses, such as the DNA-DNA hybridisation tapestry (Sibley & Ahlquist 1990) are severely limited in their taxonomic coverage. Incomplete trees are likely to yield unsatisfactory results (Isaac *et al.* 2003).

### Rates of diversification

Under an ERM model, the probability of speciation is uniform across all contemporaneous species (Raup *et al.* 1973; Raup 1985; Purvis *et al.* 1995) and is given by the equation

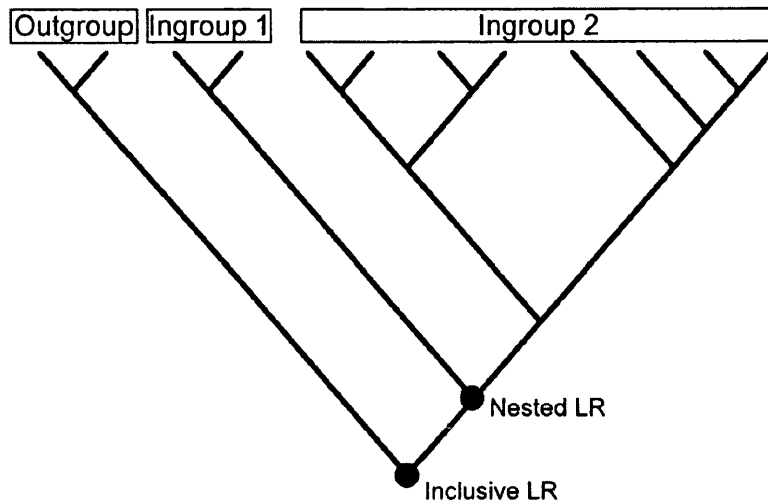
$$P = 2L / (n - 1)$$

where  $L$  is the number of species in the less diverse of two sister groups and  $n$  is the combined number of species of both sister groups (Slowinski & Guyer 1989). Note that in the case where  $L = n / 2$ ,  $P$  is set to 1 (Moore *et al.* 2004). Chan & Moore (2002) proposed two indices to extend individual node probabilities across the whole tree. These are based on the sum ( $M_{\Sigma}$ ) and the product ( $M_{\Pi}$ ) of individual node probabilities across the phylogeny (see Chan & Moore 2002, and Moore *et al.* 2004, for full details). The probability of speciation is mapped onto the phylogeny and the observed distribution of probabilities is then compared with the null distribution based on a set of trees generated under an ERM model of cladogenesis using Monte Carlo simulation. Modifications to the sum and product indices by weighting according to species diversity at each node have also been proposed ( $M_{\Sigma}^*$  and  $M_{\Pi}^*$  respectively; Moore *et al.* 2004). The main advantage of these indices over previous methods is that they avoid problems of non-independence of individual node probabilities and can account for different potential resolutions of polytomies in the phylogeny (see below; Chan & Moore 2002; Moore *et al.* 2004).

I computed both the sum and product based  $M$  indices ( $M_\Sigma$ ,  $M_{\Pi}$ ,  $M_\Sigma^*$ , and  $M_{\Pi}^*$ ) as well as two other commonly used indices ( $I_C$ , Colless 1982; Heard 1992; and  $B_I$ , Shao & Sokal 1990) to assess differences in the rate of diversification across the shorebird tree using SYMMETREE (Chan & Moore 2004). The null distribution for each statistic was generated with a sample of 100000 ERM topologies. Uncertainty associated with polytomies was assessed by generating 100000 random resolutions under a size-sensitive ERM taxon-addition algorithm (Moore *et al.* 2004). The size-sensitive algorithm provides upper and lower confidence intervals by resolving polytomies within the phylogeny to be either maximally symmetrical (lower bound of the confidence interval) or maximally asymmetrical (upper bound of the confidence interval). Within SYMMETREE these bounds correspond to the tail probabilities for the 0.025 and 0.975 frequentiles, respectively.

#### **(b) Diversification rate shifts**

I used two indices ( $\Delta_1$  and  $\Delta_2$ ) described by Moore *et al.* (2004) to identify significant shifts in the rate of diversification. The function of these indices is to estimate the probability of a shift along the lone internal branch of a three-taxon clade consisting of an outgroup sub-clade and the two most basal sub-clades of the ingroup (see fig. 1; Moore *et al.* 2004). Both indices are based on functions of two likelihood ratios, one calculated at the root of the three-taxon clade and one at the root of the ingroup sub-clade. The likelihood ratios themselves are calculated by comparing a one parameter model where the rate of diversification is constant across sister taxa (homogeneous model) with a two parameter model where the rate of diversification is greater on one side of a bipartition than on the other (heterogeneous model).



**Figure 1.** Likelihood ratios that compare a one parameter homogeneous rate model (where the rate of diversification is constant across sister taxa) with a two parameter heterogeneous rate model (where the rate of diversification is greater on one side of a bipartition than on the other) are calculated at the nested and inclusive nodes. In the figure, it appears that the rate of diversification has increased in the ingroup compared to the outgroup. However, there also appears to be an increase in ingroup 2 compared to ingroup 1. If the latter is true, then the rate increase between the outgroup and the ingroup may be artificial since it arises only because there is a later increase in the rate of diversification. An apparent rate increase at the inclusive node could therefore be due to a “trickle-down effect” (Moore *et al.* 2004). The shift indices ( $\Delta_1$  and  $\Delta_2$ ) account for the “trickle-down effect” by conditioning evidence for a change in diversification at the more inclusive node against the evidence for a change in diversification at the nested node.

The likelihood ratio statistics alone are not sufficient to identify genuine changes in the rate of diversification because an apparent change in diversification rate at the most inclusive branch of the three-taxon tree could be an artefact of a real change in diversification rate in the nested clade (see fig. 1). This is referred to as the “trickle-down effect” (Moore *et al.* 2004). To account for this, evidence for a change in diversification at the more inclusive node (i.e. the likelihood ratio estimated between the ingroup and the outgroup) must be conditioned by the evidence for a change in diversification at the nested node (i.e. the likelihood ratio estimated between the two ingroup sub-clades). Moore *et al.* (2004) propose two indices to perform this task. The first index ( $\Delta_1$ ) simply subtracts the likelihood ratio at the nested node from the likelihood ratio at the inclusive node. The value of the observed  $\Delta_1$  for the phylogeny is compared with null distribution of  $\Delta_1$  generated by Monte Carlo simulation under an ERM model of cladogenesis. The second index ( $\Delta_2$ ), adjusts the ingroup diversity to

exclude the number of ingroup species attributed to a rate increase along the internal branch. This is calculated as the total ingroup diversity minus the product of the probability of a rate shift at the internal branch, multiplied by the number of species attributable to that shift. Both  $\Delta_1$  and  $\Delta_2$  were computed using the program SYMMETREE. Note that the shift statistics were calculated simultaneously with the  $M$  indices (see above for parameter setting details). Full details of the computation of the shift statistics are provided by Moore *et al.* (2004).

### (c) *Data*

A database of behavioural, morphological, life-history, and ecological traits of shorebirds was collated from the literature using previous datasets for shorebirds as a starting point (Székely & Reynolds 1995; Reynolds & Székely 1997; Székely *et al.* 2000).

*Sexual selection and sexual conflict:* Testis size, social mating system, sexual size dimorphism, male display type, developmental mode, and parental care dimorphism were used as proxy variables for sexual selection and sexual conflict. Testis size is expected to increase with body size (Dunn *et al.* 2002) so I used a measure of relative testis size ( $\log_{10}$  [testis mass / body mass]). Testis size data was taken from Dunn *et al.* (2002). Social mating system was divided into two separate variables (male mating system and female mating system). Male social mating system was scored towards increased frequency of polygyny based on descriptions in the literature: 0, monogamous; 1, rare polygyny (<1% or limited anecdotal reports); 2, occasional polygyny (1-5%, polygyny is known to occur but it is infrequent); 3, moderate polygyny (6-20%, polygyny is well known but is not regarded as typical of the species); 4, frequent polygyny (>20%, polygyny is considered the main mating system for the species). Female social mating system was scored on the same scale but with respect to frequency of polyandry (from monogamy [0] to frequent polyandry [4]). The intensity of sexual selection is expected to increase from low in monogamous species to high in polygynous species (males) and polyandrous species (females; Andersson 1994; Székely *et al.* 2000). Sexual size dimorphism (SSD) in wing length was calculated as  $\log_{10}$  (male wing length / female wing length). Aerial acrobatic displays are associated with more intense sexual selection (Grønstøl 1996; Blomqvist *et al.* 1997). I scored male display

as ground-based (0), aerial non-acrobatic (1) and aerial acrobatic (2) following Székely *et al.* (2000). Developmental mode (the state of the chicks at hatching) acts as a constraint on the evolution of mating systems (Temrin & Tullberg 1995). Precocial chicks are able to feed themselves within hours of hatching whereas semiprecocial chicks are reliant on their parents at least until fledging. In some species, the chicks begin to feed themselves several days after hatching. Thus, I scored developmental mode as semiprecocial (0), intermediate (1) and precocial (2) for each species, based on the feeding behaviour of the chicks. Note that there are no altricial shorebirds. Finally, I used parental care data to estimate the outcomes of post-zygotic sexual conflict. This was measured as the difference in the duration of care between males and females (duration of male care – duration of female care). The scoring system for the duration of care for each sex was taken from Székely & Reynolds (1995) and Reynolds & Székely (1997).

*Life-history:* I used clutch size (as a measure of fecundity) and adult mortality as measures of life-history variation. Large clutch size and high mortality are associated with “fast” life-histories, whereas small clutch size and low mortality are associated with “slow” life-histories (Bennett & Owens 2002). Other measures of life-history, such as hatching success and fledging success, were not used because they are poorly known for many shorebird species. In addition, these traits are likely to be prone to high levels of intraspecific variation.

*Niche availability:* I used wing length (mean of male and female wing length) as an estimator of body size. Wing length is a more reliable measure of body size than, for instance, body mass, which varies greatly throughout the year, and bill length, which depends on feeding ecology. Wing length was log transformed prior to analysis. Scores of breeding habitat productivity as an estimate of potential resource availability were taken from Székely *et al.* (2004): 0, desert and semi-desert (low resource availability); 1, dry grassland, tundra, dry forest (intermediate resource availability); 2, wetland, marsh, seashore, lake, river (high resource availability). I used two measures of biogeography. First, total species range size was estimated as the difference in degrees latitude from the most northerly to the most southerly extent of the breeding ranges. Second, the breeding latitude was estimated as the midpoint between the most northerly and most southerly extent of the breeding ranges.

#### (d) *Comparative analyses*

I used phylogenetically independent contrasts through the origin for all statistical analyses of species richness (Felsenstein 1985; Harvey & Pagel 1991; Garland *et al.* 1992). Contrasts were computed using MacroCAIC (Agapow & Isaac 2002). This avoids problems of non-monophyly, non-comparability, and non-independence (Isaac *et al.* 2003). Several measures of species richness have been proposed. I used both the relative rate difference (RRD), and the proportional dominance index (PDI). RRD is given by  $\ln(S_i / S_j)$  where  $S_i$  is the species richness of the clade with the larger value of X (the independent variable), and  $S_j$  is the species richness of the clade with the smaller value of X. PDI is given by  $S_i / (S_i + S_j) - 0.5$ . Simulations show that RRD performs better than PDI when branch length information is available and the phylogeny contains at least 30 informative nodes (Isaac *et al.* 2003). PDI performs better when branch lengths are not known. Thomas *et al.* (2004) included branch length estimates but noted that these were very crude. I therefore used both RRD and PDI for all analyses. I also repeated all comparative analyses with both the estimated branch lengths, and with branch lengths set to unity. Incomplete phylogenetic data (i.e. missing taxa) can bias estimates of clade size and generate spurious results (Isaac *et al.* 2003). Missing trait data can result in some clades being excluded from analyses. Multiple-regression analyses would result in the amount of missing data increasing for each explanatory variable added since the availability of data was not uniform across all traits. To minimise bias, I therefore computed bivariate regressions of each independent variable on PDI and RRD and only performed multiple regression analyses on variables that were significant or marginally significant correlates in the bivariate analyses. I report  $r$ ,  $p$ , and  $n$ . Deviations from the assumption of Brownian motion in either the independent or the dependent variable do not seriously increase error but deviations from Brownian motion in both variables are likely to substantially increase error (Isaac *et al.* 2003). The results were consistent regardless of the measure of species richness and branch lengths used unless otherwise stated. I therefore report the results that most closely adhered to the assumptions of independent contrasts on a case-by-case basis (see Results).

I tested for correlates of species richness on the full shorebird phylogeny and then on three major monophyletic clades (see fig. 2). These were (i) the sandpipers and allies (Scolopacidae, Jacanidae, Rostratulidae, Thinocoridae, Pedionomidae); (ii) the plovers and allies (Charadriinae, Recurvirostrinae, Haematopodini, Burhinae, Chionidae,



Pluvianellidae); and (iii) the gulls and allies (Larini, Sternini, Rynchopini, Stercorariini, Alcinae, Glareolidae). This allows for the possibility that there may be different explanations for species richness in different clades. For instance, variation in social mating system is at its most extreme in the sandpipers whereas there is little variation across the gulls and allies (most species with available data are socially monogamous). Speciation in sandpipers may then be expected to vary with social mating system, but this could be clouded by the lack of variation in gulls when examined across the whole shorebird tree.

### 3. RESULTS

#### (a) *Rates of diversification*

The shorebird tree does not conform to the equal-rates Markovian model of cladogenesis. All six measures ( $B_I$ ,  $I_C$ ,  $M_\Sigma$ ,  $M_{II}$ ,  $M_\Sigma^*$ , and  $M_{II}^*$ ) indicate that there was significant variation in diversification rates across the phylogeny (table 1). These results hold regardless of whether polytomies are resolved to be symmetrical or asymmetrical.

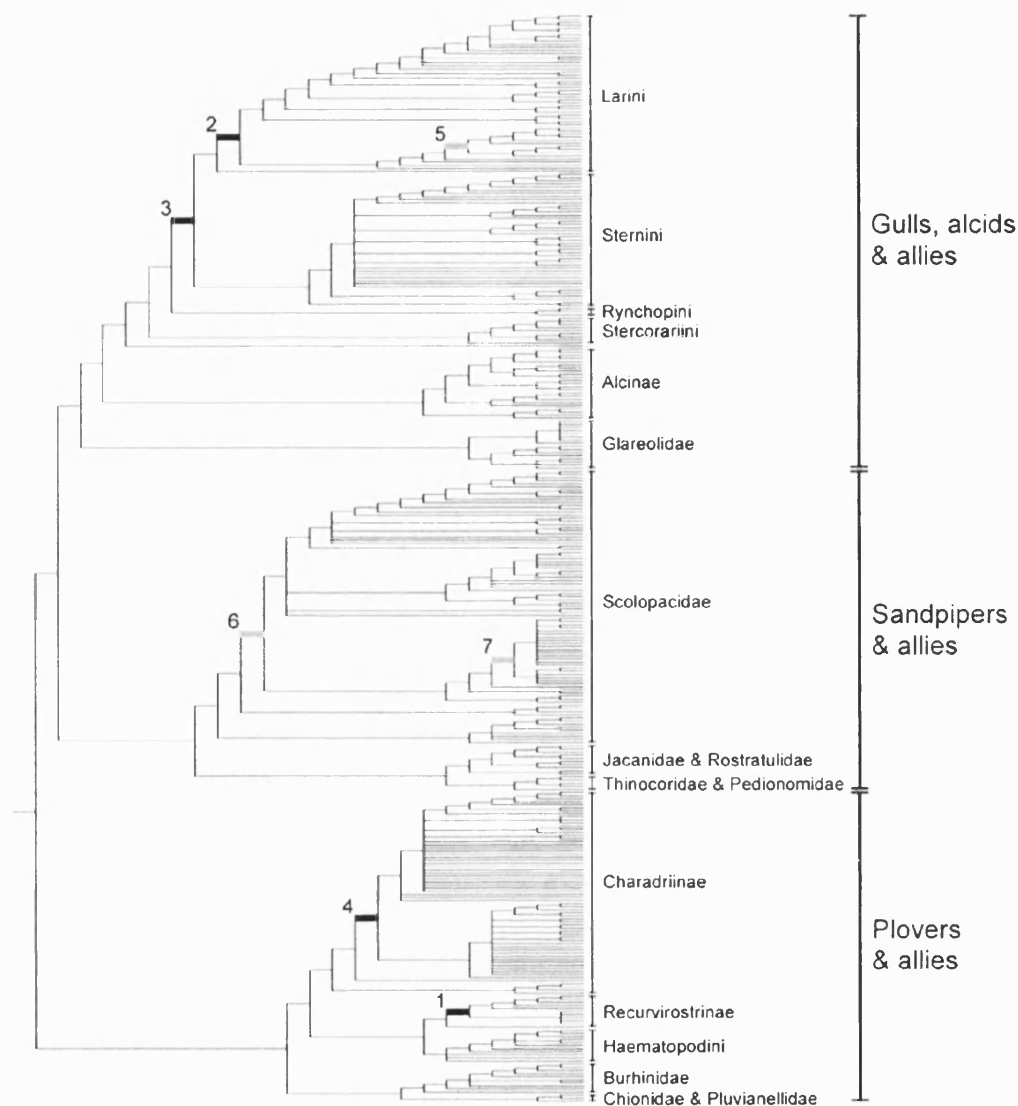
**Table 1.** Probability values corresponding to tests of ERM cladogenesis in shorebirds. All results were obtained using the SYMMETREE program (Chan & Moore 2004). The null distribution for each statistic was generated with a sample of 100000 ERM topologies for each tree size. Uncertainty associated with polytomies was assessed by generating 100000 random resolutions under the size-sensitive ERM taxon-addition algorithm, providing the upper and lower bounds of the confidence interval (Chan & Moore, 2002). The “high” and “low” values (high and low asymmetry) correspond to the tail probabilities for the .025 and .975 frequentiles, respectively. The sensitivity of the whole-tree statistics to large-scale diversification rate variation increases from left to right (i.e.  $B_I < M_\Sigma < M_\Sigma^* < M_{II} < M_{II}^* < I_C$ ).

	$B_I$	$M_\Sigma$	$M_\Sigma^*$	$M_{II}$	$M_{II}^*$	$I_C$
High	0.000	0.000	0.000	0.000	0.000	0.01685
Low	0.000	0.000	0.000	0.000	0.000	0.00017

### (b) Diversification rate shifts

There were four significant diversification rate shifts in the shorebird tree identified by  $\Delta_1$  and  $\Delta_2$  (fig. 2). These were the splits (smaller clade first) of: i) *Ibidorhyncha struthersii* from Recurvirostrinae (branch 1;  $\Delta_1, p = 0.0222$ ;  $\Delta_2, p = 0.0222$ ); ii) *Xema sabini* from Larini (branch 2;  $\Delta_1, p = 0.019$ ;  $\Delta_2, p = 0.027$ ); iii) Rynchopini from Sternini and Larini (branch 3;  $\Delta_1, p = 0.033$ ;  $\Delta_2, p = 0.037$ ); and iv) *Oreopholus ruficollis* from Charadriinae (branch 4;  $\Delta_1, p = 0.007$ ;  $\Delta_2, p = 0.011$ ).

In addition, there were three marginally non-significant diversification rate shifts (fig. 2). These were the splits (smaller clade first) of: i) *Larus philadelphia* from a clade consisting of *L. maculipennis*, *L. ridibundus*, *L. cirrocephalus*, *L. brunnicephalus*, *L. scopulinus*, *L. serranus*, *L. novaehollandiae*, *L. bulleri*, *L. hartlaubi*, and *L. genei* (branch 5;  $\Delta_1, p = 0.067$ ;  $\Delta_2, p = 0.067$ ); ii) *Limosa* from Scolopacidae, excluding *Numenius* and *Bartramia* (branch 6;  $\Delta_1, p = 0.062$ ;  $\Delta_2, p = 0.074$ ); and iii) *Lymnocyptes minimus* from *Gallinago* and *Scolopax* (branch 7;  $\Delta_1, p = 0.048$ ;  $\Delta_2, p = 0.065$ ).



**Figure 2.** The supertree of shorebirds showing significant (black bars) and marginally non-significant (grey bars) diversification rate shifts. The significant shifts were: (1) *Ibidorhyncha struthersii* from Recurvirostrinae; (2) *Xema sabini* from Larini; (3) Rynchopini from Sternini and Larini; and (4) *Oreopholus ruficollis* from Charadriinae. The marginally non-significant shifts were: (5) *Larus philadelphia* from a clade consisting of *L. maculipennis*, *L. ridibundus*, *L. cirrocephalus*, *L. brunnicephalus*, *L. scopulinus*, *L. serranus*, *L. novaehollandiae*, *L. bulleri*, *L. hartlaubi*, and *L. genei*; (6) *Limosa* from Scolopacidae, excluding *Numenius* and *Bartramia*; and (7) *Lymnocryptes minimus* from *Gallinago* and *Scolopax*.

**(c) Correlates of species richness**

*All shorebirds (table 2):* Out of 13 explanatory variables tested, more acrobatic male display behaviour was the only one associated with increased species richness (equal branch lengths, PDI:  $r = 0.211$ ,  $p = 0.042$ ,  $n = 93$  contrasts). However, this relationship was not significant with calibrated branch lengths.

*Sandpipers and allies (table 2):* Trends towards aerial acrobatic male display were associated with more speciose lineages in sandpipers (equal branch lengths, RRD:  $r = 0.381$ ,  $p = 0.024$ ,  $n = 34$  contrasts; see table 2). This was consistent with equal branch lengths and PDI and remained marginally significant ( $0.05 < p < 0.1$ ) when calibrated branch lengths were used. There were no other significant correlates of species richness amongst sandpipers. Note that the analyses of sexual size dimorphism and clutch size violated the Brownian motion assumption in both the dependent and the independent variable.

*Plovers and allies (table 2):* Trends towards more polygamous female mating systems were associated with increased species richness (calibrated branch lengths, PDI:  $r = 0.506$ ,  $p = 0.016$ ,  $n = 21$  contrasts; see table 2). This result holds for calibrated branch lengths and RRD and remained marginally significant for analyses with equal branch lengths. In addition, trends towards more precocial offspring were associated with more speciose lineages in plovers (equal branch lengths, RRD:  $r = -0.482$ ,  $p = 0.020$ ,  $n = 22$  contrasts; see table 2). This was consistent with both equal branch lengths and PDI, and calibrated branch lengths and RRD, and remained marginally significant when calibrated branch lengths and PDI were used. When both female mating system and developmental mode were included in a multivariate regression (calibrated branch lengths, PDI:  $r = 0.578$ ,  $F_{2,16} = 4.010$ ,  $p = 0.039$ ) only female mating system remained significant ( $p = 0.018$ ). The overall multiple regression model was marginally significant when equal branch lengths were used but the partial correlation of female mating system was still significant. There were no other significant correlates of species richness amongst plovers. The analysis of parental care difference violated the Brownian motion assumption in both the dependent and the independent variable.

**Table 2.** Correlates of species richness in shorebirds. All regressions were based on phylogenetically independent contrasts obtained using MacroCAIC (Agapow & Isaac 2002). Regressions that violated the assumption of Brownian evolution in the dependent variable are marked with †. Regressions that violated the assumption of Brownian evolution in the independent variable are marked with ‡. The branch length assumptions (Cal, calibrated branch lengths; Equal, equal branch lengths) and species richness index (PDI or RRD) are shown in column three. Note that the scores for female social mating system and parental care difference were constant across the gulls and allies and were therefore not included in the gulls only analyses.

	Independent variable	Taxa	Branch lengths; index	<i>n</i>	<i>r</i>	<i>p</i>
Sexual selection and sexual conflict	Relative testis size	All shorebirds	Cal; PDI	55	-0.126	0.355
		Sandpipers	Cal; PDI	24	-0.250	0.228
		Plovers	Equal; PDI	12	0.136	0.658
		Gulls	Equal; RRD	17	-0.197	0.433
	Male social mating system	† All shorebirds	Cal; PDI	95	-0.028	0.783
		† Sandpipers	Cal; PDI	30	-0.026	0.884
		† Plovers	Equal; PDI	21	0.146	0.516
		†† Gulls	Equal; RRD	44	0.154	0.323
	Female social mating system	† All shorebirds	Cal; PDI	95	-0.037	0.720
		Sandpipers	Cal; PDI	30	-0.091	0.627
		† Plovers	<b>Cal; PDI</b>	<b>21</b>	<b>0.506</b>	<b>0.016</b>
		Gulls	-	-	-	-
	Sexual size dimorphism (wing)	All shorebirds	Cal; PDI	128	-0.022	0.808
		†† Sandpipers	Cal; PDI	37	-0.101	0.546
		Plovers	Equal; RRD	26	0.270	0.174
		† Gulls	Equal; PDI	63	-0.057	0.651
	Male display type	<b>All shorebirds</b>	<b>Equal; PDI</b>	<b>93</b>	<b>0.211</b>	<b>0.042</b>
		<b>Sandpipers</b>	<b>Equal; PDI</b>	<b>34</b>	<b>0.381</b>	<b>0.024</b>
		Plovers	Equal; RRD	14	0.006	0.832
		† Gulls	Equal; PDI	43	0.100	0.518
	Developmental mode	All shorebirds	Cal; PDI	115	-0.124	0.183
		† Sandpipers	Cal; PDI	59	-0.119	0.509
		<b>Plovers</b>	<b>Equal; RRD</b>	<b>22</b>	<b>-0.482</b>	<b>0.020</b>
		†† Gulls	Equal; PDI	59	0.066	0.617
	Parental care difference	† All shorebirds	Cal; PDI	155	-0.001	0.914
		† Sandpipers	Cal; PDI	40	0.000	0.994
		†† Plovers	Equal; RRD	32	0.000	0.993
		Gulls	-	-	-	-
Life-history	Wing length	All shorebirds	Cal; PDI	128	0.045	0.615
		† Sandpipers	Equal	37	-0.136	0.415
		Plovers	Equal; RRD	26	0.254	0.201
		† Gulls	Equal; PDI	63	0.067	0.598
	Clutch size	All shorebirds	Cal; PDI	110	0.103	0.281
		†† Sandpipers	Cal; PDI	35	0.014	0.931
		Plovers	Equal; RRD	24	-0.151	0.470
		† <b>Gulls</b>	<b>Cal; PDI</b>	<b>49</b>	<b>0.278</b>	<b>0.050</b>
	Adult mortality	† All shorebirds	Cal; PDI	41	0.158	0.317
		Sandpipers	Cal; PDI	17	0.276	0.268
		Plovers	Equal; RRD	13	0.108	0.714
		Gulls	Equal; PDI	9	-0.071	0.845

**Table 2. continued.**

Niche availability	Breeding habitat	‡ All shorebirds	Cal; PDI	66	0.184	0.135
		Sandpipers	Cal; PDI	23	0.330	0.115
		Plovers	Equal; RRD	7	-0.104	0.806
		‡‡ Gulls	Equal; PDI	34	0.110	0.530
	Range size	‡ All shorebirds	Cal; PDI	115	0.047	0.615
		Sandpipers	Cal; PDI	33	0.133	0.453
		‡ Plovers	Equal; RRD	21	0.000	0.994
		‡‡ Gulls	Equal; PDI	59	0.091	0.489
	Breeding latitude	‡ All shorebirds	Cal; PDI	115	0.000	0.958
		Sandpipers	Cal; PDI	32	0.217	0.226
		Plovers	Equal; RRD	21	0.115	0.609
		‡‡ Gulls	Equal; PDI	60	-0.065	0.619

*Gulls and allies (table 2):* There were no significant correlates of species richness amongst the gulls and allies (table 2). However, clutch size was a marginally significant correlate of species richness regardless of branch length assumptions and the choice of species richness index (calibrated branch lengths, PDI:  $r = 0.278$ ,  $p = 0.050$ ,  $n = 49$  contrasts; see table 2). Two scored variables (male social mating system and parental care difference) were constant across the entire gull clade and were therefore not tested. In addition, the analyses of male social mating system, developmental mode, breeding habitat, breeding range size, and breeding latitude violated the assumption of Brownian motion in both the dependent and independent variable.

## 4. DISCUSSION

The results presented here suggest that: i) shorebirds did not speciate at random; ii) sexual selection was pervasive in driving cladogenesis in both plovers and sandpipers, although the mechanisms are likely to be different; iii) higher rates of fecundity are associated with higher species richness in gulls and allies; and iv) no single factor can explain the variation in species richness across shorebirds.

### (a) *Rates of diversification*

The degree of imbalance in the shorebird phylogeny exceeds the amount expected under a null Markovian model of cladogenesis, as indicated by all six tree imbalance indices. However, the results for  $I_C$  were slightly weaker (but still highly significant) than for the other five indices indicating that the rate of diversification varies at all levels through the

shorebird tree, but to a lesser degree towards the root (large-scale diversification rate variation increases in the following order  $B_I < M_\Sigma < M_\Sigma^* < M_{II} < M_{II}^* < I_C$ ; Moore *et al.* 2004).

Four nodes were identified where major diversification rate shifts occurred, some of which may be linked to fundamental biological traits. First, the split between *Ibidorhyncha struthersii* and Recurvirostrinae marks a transition in developmental mode in which taxa in the more species rich clade (Recurvirostrinae) are precocial whereas the more species deficient clade (*I. struthersii*) is semiprecocial (see below for further discussion). The reverse is true of the split of *Xema sabini* from Larini. Second, the rate shift between Rynchopini, and Sternini and Larini coincides with a major change in feeding apparatus and behaviour. The Rynchopini have highly specialised feeding apparatus and behaviour that may have limited opportunities to diversify, whereas the Sternini (terns) and Larini (gulls) have a range of feeding behaviours from diving to scavenging and are unlikely to be constrained in such a way. Finally, the diversification rate shift between *Oreopholus ruficollis* from Charadriinae may be spurious. Very little is known about the behaviour, ecology and life-history or phylogenetic affinities of *O. ruficollis* and its position in the shorebird supertree is based on taxonomy (Monroe & Sibley 1993). Consequently, the apparent rate shift between *O. ruficollis* and Charadriinae may be an artefact of *Oreopholus* being classified as a monotypic genus.

#### **(b) Sexual selection in plovers and sandpipers**

Sexual selection correlated with species richness in both plovers and sandpipers but the underlying mechanisms are likely to be very different. Male display type was associated with variation in species richness of sandpipers. More acrobatic displays are thought to relate to female choice whereas ground displays are associated with male-male competition (Figuerola 1999; Székely *et al.* 2000; Székely *et al.* 2004). The direction of this relationship, with increased agility associated with increased species richness, is consistent with sexual selection theory, which predicts that female mate choice can drive a runaway selection of male attractiveness traits. This is also consistent with comparative studies of birds (Owens *et al.* 1999) and agamid lizards (Stuart-Fox & Owens 2003) that found that increased sexual dichromatism (a measure of female mate choice) was associated with increased species richness. The scores of display type are

relatively crude and are likely to underestimate the full diversity of male display behaviour; the display type of few species have been analysed quantitatively. The explanatory power of display type is likely to increase with improved data resolution and I anticipate that this would result in confirmation of the conclusions presented herein.

The relationship between sexual selection and species richness in plovers is less easily explained by current theory. Evolutionary transitions towards increased polyandry were associated with increased species richness. This implies that increased sexual selection pressure on females, rather than on males, drives speciation. In general, selection on males, particularly through male-male competition or female mate choice, is cited as the most likely way that sexual selection will act on rates of speciation, however, there is no *a priori* reason why the same explanations (i.e. runaway selection) should not also be applied to male mate choice on females. Nonetheless, it is surprising that any association with polyandry should arise amongst the plovers since there are few species that are typically regarded as polyandrous (*Charadrius montanus*, *Charadrius alexandrinus*, *Eudromias morinellus*). An alternative explanation to sexual selection is that social mating system may reflect sexual conflict over mating optima (Gage *et al.* 2002). Sexually antagonistic coevolution of males and females would be expected if females are able to maximise their reproductive output by having clutches with multiple partners because males should evolve to avoid exploitation since this reduces the potential of male reproductive success. This explanation, however, seems unlikely given that no association between parental care and species richness was found.

In addition to female mating system, trends towards more precocial offspring were associated with increased species richness, although this was not significant when female mating system was accounted for. Precocial species are expected to place fewer demands on the parents in terms of the intensity of care required than semiprecocial species. This can have a knock-on effect on the rate of evolution of other phenotypic traits, notably those associated with sexual selection and sexual conflict (Thomas *et al.* unpublished data, Chapter 4). For instance, developmental mode has previously been shown to constraint social mating systems in birds (Temrin & Tullberg 1995). This raises the question: why was there no correlation between developmental mode and species richness across shorebirds as a whole? A reduction in diversification rate should



be expected in the gulls and allies since there was a switch to semiprecocial offspring at the base of this clade; this was not detected and may be explained because the gull clade is also associated with a switch to marine habitats. Whilst the demands of the young may have reduced the potential for diversification in, for example parental care and mating system, the move to marine habitats may have facilitated the invasion of many new niches. Thus, speciation may have been constrained by developmental mode but promoted by the habitat shift such that any overall change in diversification rate is undetectable.

### **(c) *Fecundity in gulls and allies***

In the absence of variation in mating system and parental care in gulls and allies, alternative factors are likely to be responsible for any variation in species richness. I found weak support for increasing clutch size as predictor of speciation suggesting that fast life-histories facilitate more rapid speciation. Support for the life-history hypothesis is weak and is absent for fecundity in most previous studies (e.g. agamid lizards, Stuart-Fox & Owens 2003; hoverflies, Katzourakis *et al.* 2001). I suggest that in the absence of more pervasive evolutionary forces life-history may influence rates of speciation but that it is relatively weak (less than 8% of the variation in species richness in gulls and allies could be explained by clutch size) and may often be obscured by such processes as sexual selection and sexual conflict.

Taken together, I have shown that in shorebirds sexual selection is a key factor in driving speciation. In sandpipers sexual selection acting on males through female mate choice is the main predictor of species richness, whereas in plovers sexual selection acting on females is associated with species richness. In the absence of expected sexual selection, increased fecundity is a weak correlate of species richness in gulls and allies. This highlights the importance of taking into consideration possible differences in the causes of speciation in monophyletic clades, even between closely related lineages.

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**Chapter 7.** *Migration route and sexual selection explain  
population declines in North American shorebirds*

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*Submitted manuscript*



## **Abstract**

Many North American shorebirds are declining. These trends reflect global patterns in shorebird populations. Here we ask what factors make some shorebird species more prone to decline than others. Specifically, we test the influence of migratory behaviour (route and distance), biogeography (population size and range), life history (body size, clutch size, mortality), and sexual selection (social mating system and testis size) on population trends in North American shorebirds. Controlling for phylogeny, we show that species that migrate across continental North America are more prone to decline than species that do not. Habitat change and increased predator numbers are plausible explanations of these declines. In addition, shorebirds that have intense sexual selection, as indicated by large testis size, are more prone to decline than species with low levels of sexual selection. This is consistent with recent comparative studies of birds and may reflect increased natural selection load on species that undergo intense sexual selection.

## 1. INTRODUCTION

Why are some species more predisposed to extinction than others? Recent estimates suggest that 1211 bird species (12% of the total) are at risk of extinction (BirdLife International 2004). The distribution of “at risk” taxa is not random, some families harbour more extinction-threatened species than other families (Bennett & Owens 1997). Rather than simply being the effect of contemporary pressures, such as habitat loss, climate change, predation, and human persecution, the risk of extinction can also be attributed to the life-history, ecology, and behaviour of a species.

Numerous hypotheses have been proposed to explain differences in extinction risk between species (Purvis *et al.* 2000; Reynolds 2003; Fisher & Owens 2004), and these can be placed into four general categories. First, extinction risk may be related to migratory behaviour. For instance, species that migrate are more prone to decline (Zöckler *et al.* 2003) or become extinct than resident species (Pimm *et al.* 1988). Second, species with small population sizes or range sizes (including island dwelling taxa) may be at greater extinction risk than those with large populations or ranges (BirdLife International 2004). Third, species with traits associated with slow life histories, such as large body size (Pimm *et al.* 1988; Gaston & Blackburn 1995) and small clutch size (Pimm *et al.* 1988), are more likely to become extinct than those with traits associated with fast life histories (Owens & Bennett 2000). Fourth, more intense sexual selection is associated with higher extinction risk (Møller 2000). Here, sexual selection pressure on species to evolve traits that enhance mating opportunities may compromise benefits normally accrued through natural selection (see Andersson 1994). In a changing environment, these sexually selected species are less able to adapt and thus are more prone to population extinction (Tanaka 1996).

Unfortunately, our understanding of what makes a species more at risk than others is limited and the few hypotheses proposed to explain extinction risk have not been rigorously tested. Furthermore, we have a poor understanding of the interactions between the intrinsic reasons that make a species *prone* to decline and the extrinsic factors that may drive decline in contemporary populations (but see Owens & Bennett 2000; Cardillo *et al.* 2004). An evaluation of the relationship between extinction risk, and population size and range is difficult, however, because measures of extinction risk

are based partly on the size and range of a species. Population trend indices, in contrast, do not depend on population or range size, and therefore allow the influence of these two factors to be assessed without fear of confounding the analyses. Whereas large-scale cross-species studies of extinction risk are effective in determining general correlates of extinction risk, studies that focus on a more limited range of taxa or restricted geographic regions are likely to be more effective at unravelling the links between intrinsic and extrinsic reasons for population decline (Fisher & Owens 2004).

Shorebirds (Aves: Charadriiformes) worldwide have suffered alarming recent declines. Forty-eight percent of 200 populations with known trends are in decline whilst only 16% are increasing (International Wader Study Group 2003). Analyses of migration monitoring data collected since 1974 (International and Maritimes shorebird surveys, Howe *et al.* 1989; Morrison *et al.* 1994) suggest that the population size of 22 species of North American shorebirds are also declining; only three species of a total of 51 populations with data were increasing (Brown *et al.* 2001; Donaldson *et al.* 2000; Alaska Shorebird Group 2004). These declines are of particular conservation concern because the reliance of shorebirds on wetland ecosystems suggests that they may be important indicators of wetland health on a global scale (CHASM 2004).

Here, we use phylogenetic comparative methods to elucidate the factors that make some North American shorebird species more prone to decline than others. The fact that shorebirds use a variety of migratory strategies, exhibit diverse life histories, and display an exceptional range of breeding behaviours (Whitfield & Tomkovich 1996; Figuerola 1999; Székely *et al.* 2004) make them an ideal group to study the effects of evolutionary predisposition on population declines. North American shorebirds are particularly good for such a study because extensive data on the biology and population trends of North American shorebirds are readily available. We ask how migratory behaviour, biogeography, life history and sexual selection influence population trends. We discuss these factors in relation to possible extrinsic threats to shorebird populations.

## 2. MATERIALS AND METHODS

We collected data on population trends, size, and range of 51 North American breeding shorebirds from the United States Shorebird Conservation Plan (USSCP; Brown *et al.* 2001) and the Canadian Shorebird Conservation Plan (CSCP; Donaldson *et al.* 2000) with additional and updated information from the Alaska Shorebird Conservation Plan (ASCP; Alaska Shorebird Group 2004). When population trends differed between the three sources, we used the most recent information. We collated data on life histories and sexual selection from the literature. The full data set is provided in Chapter 7 Appendix 1.

### **(a) *Population trends***

Scores for population trends were taken from the USSCP, CSCP, and ASCP: 1 = significant population increase; 2 = apparent population increase; 3 = stable population; 4 = apparent population decline; 5 = significant population decline.

### **(b) *Migratory behaviour***

We used both distance and route to describe migratory behaviour. Migration distance was taken as the difference in degrees latitude between the midpoint of the breeding range (estimated from the most northerly and most southerly breeding latitude) and the midpoint of the wintering range (estimated from the most northerly and most southerly wintering latitude; Hayman *et al.* 1986). Migration route was split into three dichotomous variables: (i) continental migration (i.e., migrate overland); (ii) coastal migration; and (iii) oceanic migration. Each species was assigned a score of 1 if they used the route and a 0 if they did not. A species could be scored a 1 on two different variables if they, for example, use a coastal route in one part of its range and a continental route in another. The advantage of this scoring system is that we can differentiate the effects of each route on population trends.

### **(c) *Biogeography***

Population size estimates, and scores for breeding and non-breeding distributions were taken from the USSCP, CSCP, and ASCP (Brown *et al.* 2001; Donaldson *et al.* 2000; Alaska Shorebird Group 2004). Breeding distribution followed a five-point scoring system based on the area of North America occupied by a species: 1 = more than 20%; 2

= 10-20%; 3 = 5-9.9%; 4 = 2.5-4.9%; 5 = less than 2.5%. Geographic area alone may be a poor measure of non-breeding distribution for species that disperse along a coastline, hence non-breeding distribution was scored based on measures of both area and length of coastline. Here a value of 1 = very widespread; 2 = widespread; 3 = intermediate; 4 = local; and 5 = highly restricted (see Brown *et al.* [2001] and Donaldson *et al.* [2000] for full details).

#### **(d) *Life-history***

Data on mean body mass and wing length of males and females, clutch size, and adult mortality, were obtained from the literature (see Reynolds & Székely 1997; Székely *et al.* 2000). Body mass fluctuates throughout the year so we restricted our body mass measurements to those taken during the breeding period. Literature sources are listed in Chapter 7 Appendix 1.

#### **(e) *Sexual selection***

Social mating system was used as a proxy for pre-mating sexual selection. The intensity of sexual selection is expected to be higher in polygamous than monogamous species (Székely *et al.* 2000; Dunn *et al.* 2001). Social mating system was scored dichotomously based on descriptions in the literature: 0 = social monogamy, and 1 = social polygamy by either the male or female. An alternative scoring system (0 = social polyandry, 1 = social monogamy, 2 = social polygyny) does not qualitatively alter our results. Testis mass was used as a proxy for post-mating sexual selection (i.e., sperm competition; Dunn *et al.* 2001): large testis mass is expected to reflect intense sexual selection. We used testis masses presented in Dunn *et al.* (2001). Because testis size is expected to be associated with body size, we controlled for this by including body mass in multiple regression analyses.

#### **(f) *Comparative analyses***

We first tested if the number of species with declining populations is significantly different from the number of species with increasing populations using the Wilcoxon's signed ranks test, accounting for phylogeny by means of matched-pairs comparisons. We selected pairs of species from the phylogeny that shared a unique phylogenetic history (i.e. the shared branches of each pair in the phylogeny were not also shared by another species in a separate pair) in which one species had a stable population trend and

the other was either increasing or declining. The null hypothesis is that an equal number of these pairs should be increasing and declining relative to the stable species.

We then analysed our data using bivariate and multiple regressions based on phylogenetically independent contrasts to control for the effects of shared evolutionary history (Felsenstein 1985; Harvey & Pagel 1991). Regression analyses of independent contrasts must be forced through the origin since the sign of each independent contrast is arbitrary (see Garland *et al.* 1992). Contrasts were estimated using CAIC (Purvis & Rambaut 1995). We report the Pearson correlation coefficient ( $r$ ), its significance ( $p$ ), and the number independent contrasts ( $n$ ) for the bivariate regressions, and the partial correlation coefficient ( $r_p$ ),  $F_{df\ regression, df\ error}$ , and its significance ( $p$ ), for multiple regressions. Independent contrasts are prone to deviation from the normal distribution and this can lead to errors in significance values based on parametric tests (Legendre & Desdevises submitted manuscript). We used the DOS program Multiple Linear Regression (Legendre 2002; from <http://www.fas.umontreal.ca/BIOL/legendre>) to perform permutation tests to assign significance values to all bivariate and multiple regression analyses and report only the permutation test results here. The permutation tests tended to be more conservative than their parametric equivalents, although they were qualitatively fully consistent with the parametric tests.

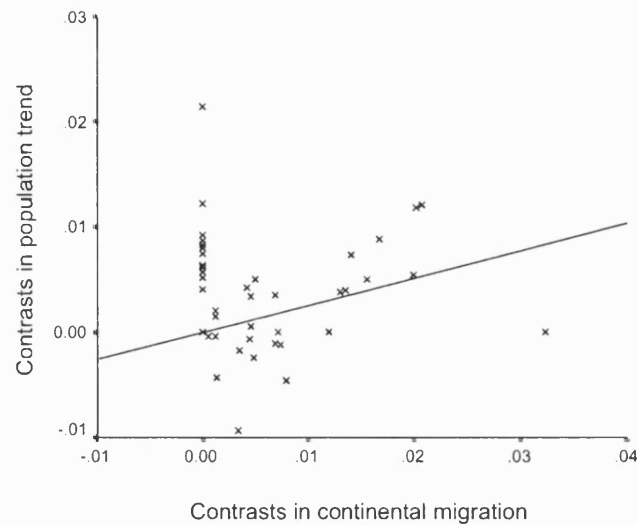
We used a recently constructed supertree of shorebirds for our comparative analyses (Thomas *et al.* 2004). We calculated contrasts among species using both the estimates of branch lengths from Thomas *et al.* (2004), and with all branch lengths set to unity. In addition, we calculated both types of contrasts for a larger dataset that included 37 subspecies listed by USSCP (Brown *et al.* 2001). Thus, we carried out two sets of analyses on the species dataset and two sets analyses of the subspecies dataset. We did not have life history or behavioural data for these subspecies and therefore could not perform all analyses using this larger data set. All data were log-transformed before analysis. Our results were consistent across the four sets of analyses except in a limited number of cases. We report the results of species level analyses with estimated branch lengths. Results from the other analyses are only included where they are not robust to phylogeny.

### 3. RESULTS

A matched-pairs comparison that accounted for phylogeny indicated 16 species pairs of North American shorebirds were declining and two were increasing (Wilcoxon's signed ranks test:  $n = 18$ ,  $Z = -3.491$ ,  $p = 0.000$ ), suggesting that significantly more shorebird species were declining than were increasing.

#### (a) *Migratory behaviour*

Species that migrate using continental routes are more likely to be declining than species that do not use continental routes ( $r = 0.374$ ,  $p = 0.012$ ,  $n = 44$ ; fig. 1). There was no relationship between a species' population trend and whether it migrated along the coast ( $r = 0.086$ ,  $p = 0.572$ ,  $n = 44$ ) or ocean ( $r = 0.053$ ,  $p = 0.730$ ,  $n = 44$ ). There was also no relationship between a species' population trend and migration distance ( $r = -0.130$ ,  $p = 0.393$ ,  $n = 44$ ). These results were consistent with a multiple regression analysis that included all migration variables ( $r = 0.458$ ,  $F_{4,40} = 2.659$ ,  $p = 0.050$ ; table 1). The overall correlation in the multiple regression analysis was marginally non-significant when using independent contrasts from the equal branch length phylogeny ( $r = 0.451$ ,  $F_{4,40} = 2.558$ ,  $p = 0.054$ ), although the partial correlations are consistent with contrasts based on estimated branch lengths.



**Figure 1.** Continental migration in relation to population trends in North American shorebirds ( $r = 0.374$ ,  $p = 0.012$ ,  $n = 44$ ). Data are phylogenetically independent contrasts with regression analyses going through the origin. Positive values on the continental migration axis indicate evolutionary trends towards a more continental migration route. Positive values on the population trend axis indicate evolutionary trends towards more severe population decline.

#### **(b) Biogeography**

The relationship between a species breeding distribution and propensity to decline was nearly significant, with larger breeding distributions associated with more severe population decline ( $r = -0.262$ ,  $p = 0.076$ ,  $n = 46$ ). This relationship was not apparent, however, when we controlled for population size and non-breeding distribution (table 1). There was no relationship between a species' population trend and size ( $r = 0.203$ ,  $p = 0.168$ ,  $n = 46$ ), nor between a species' population trend and non-breeding distribution ( $r = -0.045$ ,  $p = 0.760$ ,  $n = 46$ ).

#### **(c) Life-history**

There was no significant correlation between population trends and body mass ( $r = 0.061$ ,  $p = 0.694$ ,  $n = 42$ ), wing length ( $r = 0.079$ ,  $p = 0.612$ ,  $n = 42$ ), clutch size ( $r = 0.217$ ,  $p = 0.156$ ,  $n = 42$ ), or adult mortality ( $r = 0.050$ ,  $p = 0.804$ ,  $n = 27$ ). These results were consistent for both the bivariate and multiple regression analyses (table 1).



**Table 1.** Population trends (dependent variable) in North American shorebirds and, migration; biogeography; life history; and sexual selection. All models are based on phylogenetically independent contrasts.

Model	Explanatory variable	$r_p$	$p$
1. Migration	continental	0.302	0.005
	coast	0.061	0.557
	ocean	0.150	0.177
	distance	-0.071	0.171
2. Biogeography	population. size	0.017	0.454
	breeding range	-0.160	0.172
	non-breeding range	0.054	0.624
3. Life history	body mass	-0.253	0.353
	wing length	0.459	0.575
	clutch size	-0.368	0.586
	adult mortality	-0.054	0.575
4. Sexual selection	mating system	0.051	0.727
	testis size	0.100	0.065
	body mass	-0.127	0.241

Full multiple regression models:

1.  $r = 0.458$ ,  $F_{4,40} = 2.659$ ,  $p = 0.050$

2.  $r = 0.288$ ,  $F_{3,43} = 1.299$ ,  $p = 0.282$

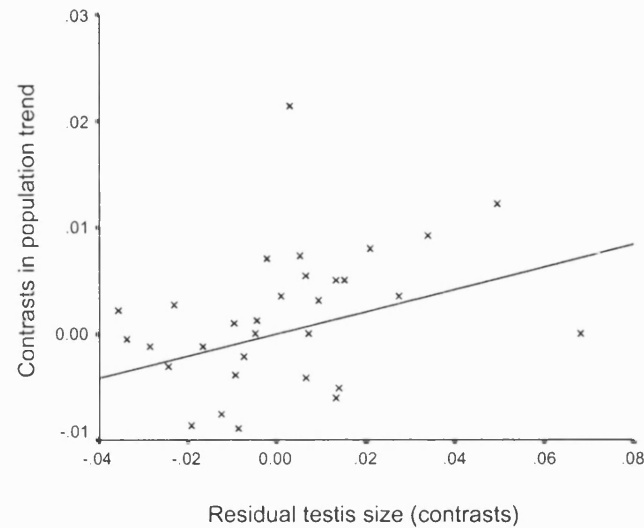
3.  $r = 0.302$ ,  $F_{4,21} = 0.527$ ,  $p = 0.701$

4.  $r = 0.369$ ,  $F_{3,27} = 1.422$ ,  $p = 0.256$

#### **(d) Sexual selection**

Partial correlation analyses (while controlling for body mass) indicated species with larger testis size were associated with more severe population decline ( $r_p = 0.106$ ,  $p = 0.044$ ,  $n = 31$ , fig. 2). However, the overall multiple regression was not significant ( $r = 0.368$ ,  $F_{2,29} = 2.277$ ,  $p = 0.121$ ), suggesting that there was a high degree of correlation among the independent variables and that the results should be treated cautiously (Zar 1998). Nevertheless, this relationship was consistent with a regression of population trend against the residuals of testis size on body mass ( $r = 0.367$ ,  $p = 0.039$ ,  $n = 31$ ), and continued to be a marginally significant predictor of population trend when the potential

effects of mating system were controlled (table 1). There was no significant relationship between social mating system and population trend ( $r = 0.071$ ,  $p = 0.645$ ,  $n = 41$ ).



**Figure 2.** The relationship between testis size and population trends in North American shorebirds ( $r = 0.367$ ,  $p = 0.039$ ,  $n = 31$ ). Data are phylogenetically independent contrasts with regression analyses going through the origin. Testis size data are based on unstandardised residuals from a bivariate regression of testis size against body mass. Note that residuals were used for illustrative purposes (see text for the results of the multiple regression analysis including testis size and body size). Positive values on the residual testis size axis indicate evolutionary trends towards a larger relative testis size. Positive values on the population trend axis indicate evolutionary trends towards more severe population decline. See the main text for the full multiple regression of population trend against testis size and body mass.

#### (e) Overall model

Finally, we included all significant and marginally non-significant ( $p < 0.1$ ) variables in a multivariate model to assess the relative importance of each explanatory variable on population trend. This included continental migration, breeding range, and testis size (table 2). In this model, we also included body mass to account for its association with testis size. Using backwards regression, the final model ( $r = 0.530$ ,  $F_{4,26} = 3.518$ ,  $p = 0.036$ ) included both continental migration ( $r_p = 0.267$ ,  $p = 0.042$ ,  $n = 30$ ) and testis size ( $r_p = 0.128$ ,  $p = 0.012$ ,  $n = 30$ ) whilst breeding range was excluded. Body mass was non-significant in the final model but was retained for the above reason.

**Table 2.** Multiple regression model of the population trend of North American shorebirds and all significant and marginally non-significant variables (continental migration, breeding range, and testis size). The model shown is based on backward regression with non-significant variables removed. Body mass is retained to control for the relationship between body size and testis size. The regression model was based on phylogenetically independent contrasts.

Explanatory variable	$r_p$	$p$
continental	0.267	0.042
testis size	0.128	0.012
body mass	-0.122	0.232

Full model:  $r = 0.530$ ,  $F_{4,26} = 3.518$ ,  $p = 0.036$

Excluded variable: breeding range

#### 4. DISCUSSION

The analyses presented here indicate that both migration route and post-mating sexual selection predict population decline in North American shorebirds. Previous studies have shown that migratory behaviour may be an important predictor of population decline (Pimm *et al.* 1988; Prinzing *et al.* 2002). However, to our knowledge, this is the first cross species study to explicitly show a link between the route a species migrates and the likelihood of it declining.

Severe population declines were strongly associated with species that migrate along continental routes. This result was robust to both sets of branch length assumptions and was independent of migration distance, body size, and post-mating sexual selection. Despite this apparently strong relationship, only a small (14%) percentage of the variation in population trends among species contrasts was explained by the continental route variable. This lack of explanatory value might be due to two reasons. First, the data on migration route were relatively crude. For example, we clumped all species that migrate continentally together, despite the fact that these species use different flyways and patterns of movement (e.g. narrow band, widespread, narrow band and widespread, jumps, and crossband, see Skagen *et al.* 1999). We predict that by increasing the resolution of our data the relationship between migration routes and population trends is

likely to improve. Additional noise could have also been added by using species that exhibit multiple migration routes (e.g. American Golden-plovers *Pluvialis dominica* migrate continentally during northward migration but over the ocean during southward migration), or from including species that migrate over a broad range even though their predominant route was continental. Second, it is likely that a variety of intrinsic (biological) and extrinsic (habitat loss, climate change, predation, persecution) factors are influencing population trends, so finding any one variable that explains a large amount of the variation would be difficult.

We suggest two explanations for the relationship between population trends and continental migration. First, continental migrants may be declining because of large-scale habitat change. In contrast to coastal migrants, many of the shorebirds that migrate along the continental interior flyways rely on small, often ephemeral, wetlands scattered over a large area (Skagen & Knopf 1993; Skagen 1997; Brown *et al.* 2001). These sites are seldom recognized by the Western Hemisphere Shorebird Reserve Network and Important Bird Areas initiatives, which seek to highlight and thereby protect larger stopover sites (Myers *et al.* 1987, see [http://www.audubon.org/bird/iba/iba\\_intro.html](http://www.audubon.org/bird/iba/iba_intro.html)), and have been lost extensively during the past and present centuries (Senner & Howe 1984). Indeed, the conversion of upland areas (along with suppression of fire) into agriculture is one of three primary reasons for the extinction of the Eskimo curlew (*Numenius borealis*), a continental migrant whose numbers plummeted during the late 1800s and early 1900s (Gill *et al.* 1998). Loss of ephemeral wetland habitats as an explanation for shorebird declines clearly warrants further investigation.

Second, continental migrants may be declining due to recent increases in predator numbers. Predation pressure may be especially acute when individuals aggregate at migratory staging and stopover sites (Reed 1999). This threat seems particularly relevant given the widespread increases in raptor numbers across continental North America between the late 1970s and the late 1990s (Hoffman & Smith 2003). It seems unlikely, however, that enhanced predator numbers are the reason for declines in continental migrants. Raptor numbers are increasing across the continent (Hoffman & Smith 2003), making declines in continental and coastal migrants equally likely. Detailed studies on western sandpipers (*Calidris mauri*) indicate the situation is even

more complex. In contrast to the expected relationship, Ydenberg *et al.* (2004) suggested that enhanced predator numbers were actually misleading biologists into thinking shorebirds were declining. They showed that the length of stay of western sandpipers at stopover sites on the Strait of Georgia actually decreased with increasing predator numbers. This had the effect of making the population appear to be declining when in reality the species was simply migrating through the area faster. The role of predation as an explanatory factor for shorebird declines requires further investigation, both at the intra- and inter-species level.

In addition to migration route, our analyses suggest that species with intense post-mating sexual selection (i.e. large testis size) are more likely to decline. Morrow & Pitcher (2003) also found that large testis size was associated with heightened extinction risk across all birds. In a similar analysis restricted to European birds, Prinzing *et al.* (2002) found no relationship between sexual selection and population decline. However, the latter study did not include any measure of post-mating sexual selection, instead relying on plumage dimorphism and sexual size dimorphism (SSD) as proxies for sexual selection. We offer two possible explanations for the disparity in results between studies that investigated pre- and post-mating sexual selection effects on extinction risk. First, measures of pre-mating sexual selection are too crude or do not reliably reflect the intensity of sexual selection. For example, our relatively simplistic scoring of social mating system likely fails to capture the full variation of sexual selection in shorebirds. In addition, SSD may not be a good indicator of sexual selection since sexual selection may either select for small or large size in males depending on the type of male display (Székely *et al.* 2000). Second, the selection costs to a species as a whole for evolving sperm competition abilities (post-mating sexual selection) may be substantially higher than the costs to a species for developing traits that attract mates or enhances competition with other males (pre-mating sexual selection). Whereas mate choice and competition predominantly alters sexual selection load on males, sperm competition acts both on males and females (see Briskie *et al.* 1997). The increased sexual selection load is expected to be important in a changing environment since sexually selected species are likely to be less able to adapt than other species (Tanaka 1996). However, our analyses do not allow us to identify the environmental change that may ultimately be the driving factor behind population decline associated with sexual selection. It is therefore desirable that future comparative and modelling studies address the interactions between

post-mating sexual selection and environmental variability, in particular the effects of anthropogenic change (e.g. habitat loss, climate change, persecution, introduced predators) on population decline and extinction.

Taken together, our analyses indicated that North American shorebirds that use continental migration routes and have high post-mating sexual selection pressures are more likely to decline. Thus, our results support Morrow & Pitcher (2003) in highlighting the role of sexual selection in the decline of species. Further study is required to test whether this relationship holds for shorebirds globally and for migratory birds in general.

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## Appendix 1.

Scores for population trend, migratory behaviour, biogeography, life history, and sexual selection for 51 North American shorebird species. All data on population trends, breeding distribution, non-breeding distribution, and population size were taken from references 1-3. Migration route scores (coastal migration, continental migration, and oceanic migration) were based on data and descriptions in references 1-3, individual Birds of North America reports were also used where necessary. Testis size data were from reference 4. Migration distances were estimated based on range maps in reference 7. References for mating system, body mass, wing length, clutch size, and adult mortality listed in last column. See text for explanation of scoring for each variable. In addition, the pairs of species used for matched pairs comparisons are included in the second column (see text for details).

Species	Pair	Population trend	Breeding distribution	Non-breeding distribution	Population size	Coastal migration	Continental migration	Oceanic migration	Log testis size	Migration distance	Mating system	Body mass	Wing length	Clutch size	Adult mortality	References for mating system, body mass, wing length, clutch size, and adult mortality
<i>Aphriza virgata</i>	c	4	4	3	70000	1	0	0		23.5	0	201.55	175.3	4		5; 5; 5; 6; #
<i>Arenaria interpres</i>	a	4	2	2	235000	1	0	0	-0.69897	59.5	0	110.5	156	3.5	33.6	5; 5; 5; 12; 7
<i>Arenaria melanocephala</i>	a	3	5	3	80000	1	0	0		19.5	0	118.9	145.25	4	19.5	6; 8; 9; 15; 10
<i>Bartramia longicauda</i>	1	2	2	3	350000	0	1	0		78.5	0	142.1	168	4		6; 6; 6; 6; #
<i>Calidris alba</i>	f	5	2	1	300000	1	1	0	0.29225607	71	0	54.1	127.5	3.9	44	5; 5; 5; 12; 7
<i>Calidris alpina</i>	b	5	2	3	1525000	1	1	1	0.2121876	27	0	43.05	114	3.9	26	5; 5; 5; 11; 11
<i>Calidris bairdii</i>	b	3	3	3	300000	0	1	0	-0.24413	112	0	39.5	127	4		5; 5; 5; 15; #
<i>Calidris canutus</i>	c	3	3	3	400000	1	0	1	0.31597	71.5	0	137	171	3.7	32.4	5; 5; 5; 12; 12
<i>Calidris fuscicollis</i>	d	3	3	3	400000	0	1	1	-0.1739252	107	1	42.75	123.5	4		5; 5; 5; 15; #

<i>Calidris maritima</i>	g	2	3	3	15000	1	0	0	-0.22914799	15	0	71.95	129.5	3.9	57	5; 5; 5; 12; 13
<i>Calidris mauri</i>	e	3	4	2	3500000	1	0	0	-0.30980392	48.5	0	29.5	99.05	3.9	41.75	5; 5; 5; 33; 11
<i>Calidris melanotos</i>	f	3	2	3	400000	0	1	0	0.31597035	91.5	1	81.45	137.5	4		5; 5; 5; 15; #
<i>Calidris minutilla</i>	d	5	2	2	600000	0	1	1	-0.85387196	48.5	0	21.25	90.7	3.9	48.5	5; 5; 5; 15; 11
<i>Calidris ptilocnemis</i>	g	3	5	4	150000	1	0	0	0.31597035	9.5	0	79.65	123.4	4		5; 5; 5; 15; #
<i>Calidris pusilla</i>		5	3	3	3500000	1	1	1	-0.537602	65	0	26	98	4	42.5	5; 5; 5; 15; 11
<i>Catoptrophorus semipalmatus</i>	h	3	3	3	250000	1	1	1		20.5	0	287.2	209.35	4	14	14; 15; 15; 15; 6
<i>Charadrius alexandrinus</i>		5	3	4	15700	1	1	0		3.5	0	47.65	111.5	3	38.2	5; 5; 5; 12; 7
<i>Charadrius hiaticula</i>	i	3	5	3	10000					-9					13.4	#; #; #; #;
<i>Charadrius melodus</i>	i	5	4	4	5970	1	1	0		16.5	0	53	120.5	3.3	34.5	6; 6; 6; 6; 13
<i>Charadrius montanus</i>	j	5	5	4	9000	0	1	0	-0.40893539	11.5	1	108	147.5	3		16; 15; 15; 15; #
<i>Charadrius semipalmatus</i>	j	3	1	1	150000	1	1	0	-1	67	0	46.95	124.65		40	6; 6; 6; #; 6
<i>Charadrius vociferus</i>	k	5	1	2	1000000	0	1	0	-0.7447275	8.5	0	96.55	167	4		17; 12; 12; 15; #
<i>Charadrius wilsonia</i>	k	3	4	3	6000	1	0	0		9.5	0	61	117	3		12; 15; 15; 15; #
<i>Gallinago gallinago</i>	r	5	1	2	2000000	1	1	1		28	0	119.5	134	3.9	58.3	5; 5; 5; 12; #
<i>Haematopus bachmani</i>	l	3	3	4	8900	1	0	0	-0.31875876	0	0	648	246.25	2	10	15; 18; 19; 20; 6
<i>Haematopus palliatus</i>		3	3	4	7500	1	0	0	-0.50863831	2.5	0	602.5	256.95	2.6	15	15; 18; 19; 34; 13
<i>Himantopus mexicanus</i>		3	1	2	151600	0	1	0		.	0	168.65	217.1	4		21; 6; 30; 6; #

<i>Limnodromus griseus</i>		5	3	2	320000	1	1	0	-0.7212464	39	0	113.5	144.5	4.1		15; 12; 12; 15; #
<i>Limnodromus scolopaceus</i>		2	4	3	500000	0	1	0	-0.79588002	34.5	0	104.5	143.5	3.9		22; 12; 12; 6; #
<i>Limosa fedoa</i>	m	4	3	3	171500	1	1	0	-0.39794001	25	0	370.5	234.4	4.1	4	23; 15; 15; 15; 6 15; 15; 15; 35; #
<i>Limosa haemastica</i>	m	3	5	5	50000	1	0	0	-0.76955108	100.5	0	255.5	213.2	4	12	12; 12; 12; 6; 6
<i>Limosa lapponica</i>		3	4	3	100000	0	0	1	-0.7447275	63.5	0	333.5	216.5	3.72	12	5; 5; 5; 7; 11 6; 15; 15; 15; 11
<i>Micropalama himantopus</i>		3	3	3	200000	0	1	0		66.5	0	58.1	133	3.9		#; #; 6; 6; # 12; 12; 12; 12; 7
<i>Numenius americanus</i>	n	5	3	3	20000	1	1	0		15	0	699.35	285.3	4	30	24; 15; 15; 15; 25 15; 15; 15; 12; 13
<i>Numenius borealis</i>	o	5	5	5	50	0	1	1		101			207	4		5; 5; 5; 12; 11 26; 15; 15; 15; #
<i>Numenius phaeopus</i>	n	3	3	3	57000	1	1	1	-0.45593196	68	0	383	246.5	3.9	30.8	6; 6; 31; 6; 13 12; 19; 19; 12; 6
<i>Numenius tahitiensis</i>	o	3	5	3	10000	0	0	1		68.5	0	433.5	237.5	4	85	27; 15; 15; 15; #
<i>Phalaropus fulicaria</i>	h	4	1	3	1000000	0	0	1	-0.0222764	78.5	0	55.9	133	3.8	91	5; 5; 5; 29; 13 28; 15; 15; 15; #
<i>Phalaropus lobatus</i>		4	2	1	2500000	1	1	0	-0.85387196	62	0	34.9	111	4	54	
<i>Pluvialis dominica</i>	p	5	2	3	150000	0	1	1	-1.09691001	89.5	0	145.5	182	4		
<i>Pluvialis fulva</i>	p	3	5	4	16000	0	0	1		73	0	135	167.3	4	20.2	
<i>Pluvialis squatarola</i>		3	2	3	200000	1	1	1	-1.30103	60	0	227.2	198	4	14	
<i>Recurvirostra americana</i>		3	2	3	450000	1	1	0		16.5	0	316.6	220.05	3.7		
<i>Scolopax minor</i>		5	2	3	5000000	0	1	0	-0.06048	4.5	0	189.45	135	3.9	57.75	
<i>Steganopus tricolor</i>		5	2	5	1500000	0	1	1	-0.25963731	72.5	1	59.15	130.5	4		

<i>Tringa flavipes</i>	r	5	2	1	500000	1	1	0	-0.60205999	67.5	0	81.85	161	4		32; 12; 12; 6; #
<i>Tringa incana</i>		3	3	2	10000	1	0	0		65		108.5	164			#; 31; 31; # #
<i>Tringa macularia</i>	q	3	1	1	150000	1	1	1	-0.25963731	44	0	42.45	107	4	47	5; 5; 5; 12; 11
<i>Tringa melanoleuca</i>	r	3	2	1	100000	1	1	0	-0.58502665	61.5	0	170	197.5	3.7		32; 6; 12; 6; #
<i>Tringa solitaria</i>		3	3	2	25000	1	1	0	-1	66	0	48.6	133.5	4		6; 12; 12; 6; #
<i>Tryngites subruficollis</i>	e	4	3	4	15000	0	1	0	0.50242712	100	1	61.75	132.5	4		5; 5; 5; 15; #

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## **Appendix 1. *Publication bias in waders***

Gavin H. Thomas, Tamás Székely, and William J. Sutherland (2003)

***Wader Study Group Bulletin***, 100: 216-223

# Publication bias in waders

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Why are some waders better studied than others? We investigated the effects of life-history and ecological traits (population size, conservation status, body mass, wing length, breeding latitude, mating system, and migratory behaviour) on the number of publications in waders. A phylogenetic comparative approach is employed using an unpublished wader supertree. Overall, population size appears to be the most useful predictor of citation. The presence of publication bias may impact upon comparative and meta-analyses. The trend towards studies of taxa with large populations indicates that taxa at risk of local or global extinction may be understudied.

## INTRODUCTION

Why do we know more about some waders than others? Oystercatchers, for instance, are popular study organisms of ecologists, whereas other waders such as the magellanic plover are very little studied. We might predict that those taxa that are more common, or that are perceived to have greater evolutionary interest are likely to have a more extensive record in the scientific literature. Some might consider polygynous or polyandrous species to be more intriguing than their monogamous contemporaries, and thus polygynous species may have a greater than expected presence in the literature. Similarly, wader enthusiasts may be more intrigued by migratory species than non-migratory ones.

Publication biases are frequently discussed in the scientific literature (e.g. Dubois & Cezilly 2002, Jennions & Møller 2002). Particular interest has been aroused amongst palaeontologists for whom the quality of the fossil record is a major concern. For example, Koch (1978) demonstrated a trend towards studies of common and biostratigraphically important taxa. Such biases have led to the suggestion that estimates of the diversity of the fossil record are unreliable and are a reflection of the endeavour of systematists rather than a reliable indicator of any biological trend (Sheehan 1977).

It is not yet clear what manner of bias exists across wader studies, nor is it obvious what impact this may have on our understanding of their biology. The focus of our study is therefore to investigate publication bias in waders with respect to a range of life-history and ecological traits, namely, population size, conservation status, body mass, wing length, breeding latitude, mating system, and migratory behaviour. We aim to quantify some of the key variables that may influence the choice of study taxa and present statistical analyses using the method of phylogenetically independent contrasts (Felsenstein 1985, Harvey & Pagel 1991).

## MATERIALS AND METHODS

### Data and phylogeny

Published wader studies were identified from online searches of Web of Science (WoS). Both the text (i.e. title, abstract and keywords), and title-only options of WoS were searched using the names of 221 species of waders and 16 species of sandgrouse (all of which were included as an outgroup) to give two measures of the publication record of each species since 1980 (the full date range covered by WoS; see Appendix 1). Species names were taken from Monroe & Sibley (1993).

Estimates of population size were taken from del Hoyo *et al.* (1996). Only those taxa for which an estimate of the worldwide population (as opposed to regional or local estimates) is provided, or can be readily calculated, were used.

Conservation status was scored using the following categories listed in Stattersfield & Capper (2000): not globally threatened (1); least concern (2); near threatened (3); conservation dependent (4); vulnerable (5); endangered (6); critically endangered (7); extinct in the wild (8); extinct (9). Of the 237 species considered in this study, 236 fell into one of these nine categories, and only one (*Glareola nordmanni*) is listed as data deficient.

Data for the remaining variables – body size (body mass and wing length), breeding latitude, mating system, and migratory behaviour – are taken from the data sets of Reynolds & Székely (1997), and Székely *et al.* (2000). We used the mean values of body mass and wing length rather than splitting these measurements by sex. Breeding latitude was sub-divided into two variables. Absolute breeding latitude is a measure of the distance (in degrees latitude) of the breeding site from the equator (see Reynolds & Székely 1997). A categorical variable of breeding site was used to split waders into those breeding north of the equator (scored as 1) and

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**Table 1.** Univariate regression of population size (pop), conservation status (con), body mass (mas), wing length (win), absolute breeding latitude (lat), migratory distance (mig), and mating system (mat) against the number of citations in title-only, and text.

	Title-only			Text		
	<i>r</i>	<i>F</i>	<i>P</i>	<i>r</i>	<i>F</i>	<i>P</i>
Pop	0.244	5.135	0.026	0.346	10.999	0.001
Con	-0.126	3.617	0.058	-0.196	8.897	0.003
Mas	0.129	2.528	0.114	0.175	4.766	0.031
Win	0.071	0.914	0.340	0.060	0.640	0.425
Lat	0.281	9.266	0.003	0.278	9.074	0.003
Mig	0.369	17.296	0.000	0.371	17.575	0.000
Mat	-0.111	1.823	0.179	-0.136	2.754	0.099

those breeding south of the equator (scored as 0). Social mating system was first collated as a single variable with three categories (polygyny, 1; monogamy, 2; polyandry, 3), and then as a set of two dummy variables each with two categories (Zar 1996): dummy variable 1 consisted of one category for polygynous taxa (1) and one category for monogamous or polyandrous taxa (2). Conversely, dummy variable 2 consisted of one category for polyandrous taxa (1) and one category for monogamous or polygynous taxa (2). The function of the dummy variables was to separate the effects of interest in male-based sexual selection (dummy variable 1) from those in female-based sexual selection (dummy variable 2, Székely *et al.* 2000). Migratory behaviour is the migratory distance measured in degrees latitude between the breeding and wintering ranges (see Reynolds & Székely 1997).

The phylogeny (not shown) is an unpublished supertree of waders incorporating the same 237 species as our data set (see Sanderson *et al.* 1998 for a review, and Pisani *et al.* 2002 for a recent practical application of supertree methods). The wader supertree supports the monophyly of the two major lineages (Scolopacida and Charadriiida), and as such follows the main conclusions of established phylogenetic hypotheses (e.g. Strauch 1978, Sibley & Ahlquist 1990, Chu 1995). It

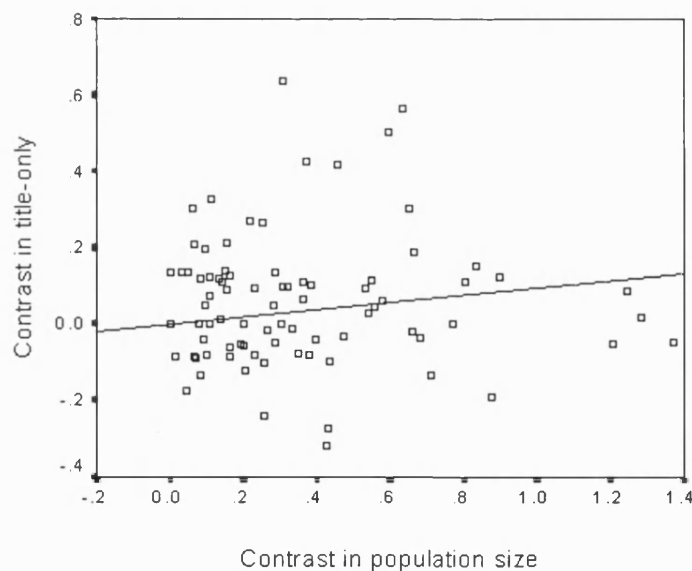
also has the distinct advantage of covering the entire taxonomic range of the waders.

### Phylogenetic analyses

Comparative analyses of publication bias were carried out using Felsenstein's (1985) method through the evaluation of phylogenetically independent contrasts for all variables as implemented by CAIC (Purvis & Rambaut 1995). This method incorporates phylogenetic history into statistical analyses to prevent the inflation of the degrees of freedom that arises from the use of non-independent samples (Harvey & Pagel 1991).

We considered conservation status and mating system as continuous variables because they both represent a gradation from one extreme to another. Conservation status can be thought of as a continuum from not threatened (1) to extinct (9). Similarly, the three categories of social mating system (polygyny, 1; monogamy, 2; polyandry, 3) can be regarded as a continuum of intensity of sexual selection on males from most (social polygyny) to least (social polyandry).

All variables were logarithmically transformed prior to calculation of independent contrasts ( $\log_{10} x + 1$ ). We used the

**Fig. 1.** Regression through the origin between population size contrast and title-only contrast. All data were  $\log_{10}(x+1)$  transformed prior to calculation of contrasts.

**Table 2.** Multivariate regressions with title-only as the dependent variable (definitions as for Table 1).

Model	Title-only			Significant predictors in model
	$r^2$	$F$	$P$	
1 (pop, con, mas, win, mig, lat, mat)	0.401	2.864	0.021	pop
2 (pop, con, mas, win, mig, lat)	0.400	3.445	0.010	pop
3 (pop, con, mas, win, lat)	0.393	4.136	0.005	pop
4 (pop, con, mas, win)	0.391	5.287	0.002	pop
5 (pop, con, mas)	0.348	6.058	0.002	pop
6 (pop, con)	0.337	8.915	0.001	pop
7 (pop)	0.277	3.921	0.001	pop

Crunch option of CAIC, allowing comparisons of all nodes in the tree. Branch lengths were not known for many nodes, thus they were set to unity.

Univariate regressions of citations were performed using population size, conservation status, body mass, wing length, absolute breeding latitude, migration distance, and mating system, respectively as the independent variable. Multivariate regressions including all of the above independent variables in the initial model were performed. Following this, we used the backward regression approach whereby the variable with the weakest correlation was removed from the model systematically until all variables remaining in the final model have a significant correlation with the dependent variable. In addition, we carried out multivariate regressions using the two dummy variables described above for mating system. All regressions (uni- and multivariate) were carried out twice, using either the number of citations in title-only, or the number of citations based in text, as the dependent variable. Univariate and multivariate regressions between phylogenetic contrasts were forced through zero (Harvey & Pagel 1991, Garland *et al.* 1992). We report the correlation coefficient,  $r^2$  or  $r$ , and  $F_{df\text{ regression}, df\text{ error}}$ . Finally, the binomial test was used to investigate any trend in the levels of citation between taxa breeding north (scored as 1) or south (scored as 0) of the equator.

## RESULTS

### Univariate analyses

Citations in title-only significantly correlated with population size (Table 1, Fig. 1;  $r^2 = 0.060$ ,  $F_{1,81} = 5.135$ ,  $P = 0.026$ ), absolute breeding latitude (Table 1;  $r^2 = 0.079$ ,  $F_{1,108} = 9.266$ ,  $P = 0.003$ ), and migration distance (Table 1;  $r^2 = 0.136$ ,  $F_{1,110} = 17.296$ ,  $P = 0.000$ ). These results suggest that those taxa that have larger population sizes, live further from the equator, and migrate furthest, are more likely to be studied than those that have small populations, live on or around the equator, and do not migrate. In addition, there were no further significant correlations with the remaining independent variables (Table 1).

The results in citation in text and population size (Table 1, Fig. 2(a);  $r^2 = 0.120$ ,  $F_{1,81} = 10.999$ ,  $P = 0.001$ ), absolute breeding latitude (Table 1;  $r^2 = 0.078$ ,  $F_{1,108} = 9.074$ ,  $P = 0.003$ ), and migration distance (Table 1;  $r^2 = 0.138$ ,  $F_{1,110} = 17.575$ ,  $P = 0.000$ ) were consistent with the title-only data. In addition, significant correlations were also found between citations in text and conservation status (Table 1;  $r^2 = 0.038$ ,  $F_{1,223} = 8.897$ ,  $P = 0.003$ ), and body mass (Table 1, Fig. 2(b);

$r^2 = 0.031$ ,  $F_{1,150} = 4.766$ ,  $P = 0.031$ ). Conservation status had a negative correlation ( $B = -0.635$ ) and is also highly consistent with population size in indicating that the most common taxa (conservation status of 1) are more likely to be studied than are rare taxa. The trend in body mass is towards an increase in citation with increasing mass. Given this relationship, it is perhaps surprising that no such relationship was found with wing length. Mating system showed no significant correlation with text (Table 1).

### Mating system

Differences in mating system (male driven or female driven) were not correlated with citations in title-only or in text in a multivariate model using the two dummy variables as independent variables (title-only,  $r^2 = 0.024$ ,  $F_{2,145} = 1.807$ ,  $P = 0.168$ ; text,  $r^2 = 0.027$ ,  $F_{2,145} = 2.001$ ,  $P = 0.139$ ). This supports the univariate analysis in suggesting that mating system has not been a major factor in determining the choice of study taxon in waders (Table 1).

### Breeding latitude

Regression analysis of absolute breeding latitude revealed a strong correlation with both title-only and text, indicating that waders breeding away from the equator are more often studied. We carried out a binomial test to determine whether this was driven by any trend favouring taxa north or south of the equator (66 positive contrasts, 42 negative contrasts,  $n = 108$  contrasts,  $p = 0.027$ ). Taken together, the results of the regression analysis and of the binomial test, suggest that waders breeding north of the equator are more often studied than those that breed south of the equator.

### Multivariate analysis

The initial multivariate model included population size, conservation status, body mass, wing length, absolute breeding latitude, mating system, and migratory distance regressed first against title-only (Table 2), and then against text (Table 3). These models both explained a significant amount of variation in contrasts of title-only ( $r^2 = 0.401$ ,  $F_{7,30} = 2.864$ ,  $P = 0.021$ ) and of text ( $r^2 = 0.478$ ,  $F_{7,30} = 3.921$ ,  $P = 0.004$ ). To determine the minimum possible number of significant predictor variables, we took a backward regression approach (see Methods). Only population size remained in the final model with title as the independent variable (Table 2;  $r^2 = 0.277$ ,  $F_{1,36} = 3.921$ ,  $P = 0.001$ ; see also Fig. 1). The final model with text as the independent variable contained

**Table 3.** Multivariate regressions with text as the dependent variable (definitions as for Table 1).

Model	Text			Significant predictors in model
	$r^2$	$F$	$P$	
1 (pop, con, mas, win, mig, lat, mat)	0.478	3.921	0.004	pop, mas
2 (pop, con, mas, win, mig, lat)	0.478	4.726	0.002	pop, mas, win
3 (pop, con, mas, win, mig)	0.476	5.822	0.001	pop, mas, win
4 (pop, mas, win, mig)	0.467	7.238	0.000	pop, mas, win
5 (pop, mas, win)	0.460	9.648	0.000	pop, mas, win

population size, body mass, and wing length (Table 3;  $r^2 = 0.460$ ,  $F_{3,34} = 9.648$ ,  $P = 0.000$ ; see Fig. 2 for univariate regressions). Taken together, the results presented herein suggest that population size is the major variable in predicting citations in studies of waders.

## DISCUSSION

The emergence of population size as a key variable in predicting citation level in waders is largely expected. Where a species is numerous, field studies are likely to be more efficient in terms of data collection and the results more robust due to increased sample size purely because increased numbers should make observation easier. Hence, whilst species that are globally threatened may be of more intrinsic interest from a conservation perspective, they appear to be less likely to be well studied than non-threatened taxa.

The problem of bias in the fossil record (Koch 1978, Sheehan 1977) may not be directly related to typical studies of waders, however the underlying causes are arguably similar. Abundance of suitable rock outcrops, and geographic factors are cited as major factors that drive systematic bias (Raup 1976). The abundance of fossiliferous rocks is directly analogous to population size because both can be linked to ease of study. The well-documented trend towards palaeontological studies at North American and Western European sites (Smith 2000) may be regarded as funding or politically driven. Whilst our results intimate that waders breeding in the northern hemisphere are more frequently studied than their southern hemisphere counterparts, we need additional geographic data to support or refute this claim.

We should not be surprised by correlative trends with citation of several other variables if their relationship with population size is accounted for. Gaston & Blackburn (1996) discuss the interrelationships of abundance, geographic range, and body size. Specifically, they highlight the notion that large species are typically less abundant than small-bodied ones. With this in mind we would predict that small taxa are likely to be studied (and therefore cited) more often than are large taxa. However, in waders we have demonstrated that the reverse may be true. We cautiously suggest that this may be for reasons of practicality as larger species are easier to observe. This implies that to determine the easiest taxon to study, there is a trade off between population size and body size. The results of the multiple regression against citations in title-only had only population size in the final model (Table 2) indicating that population size is a more useful predictor, although both body mass and wing length were present alongside population size in the final model with text as the dependent variable (Table 3). However, ease

of observation may not be a function of population or body size alone, and many other factors such as habitat and behaviour may yet alter these conclusions.

It is apparent that population size alone cannot explain all of the variation in citation rate of wader studies. The possible relationships between predictor variables are multifarious and disentangling these from each other confounds interpretation of their individual and collective impact on wader citation. For example, migratory species are generally confined to temperate zones (Bennett & Owens 2002), where we also expect larger taxa. However, it is clear that when all variables are controlled for, population size is the only variable that consistently correlates with citation (both title and text), and on this basis we cautiously suggest that it is the predominant factor in guiding the choice of study taxon amongst wader workers.

Aside from recognising those factors that influence our choice of study system, it is also important to consider how this affects our understanding of wader biology. In direct response to this, two key questions arise. First, how does bias impact upon our interpretation of data from wader research? And second, what are the major gaps in our knowledge of waders? We can further disseminate these questions by thinking of waders first in the context of the group as whole and in particular of those studies that are concerned primarily with evolutionary questions (frequently using literature based comparative or phylogenetic comparative methods), and second, those studies focusing on particular aspects of behaviour, ecology, or conservation in individual taxa that may involve direct observations or manipulations (field or laboratory methods).

Publication bias has only recently become a major concern in ecology and evolution (see Møller & Jennions 2001 for a review) but the implications for analyses of biased data are more firmly established. Much of the literature is based around the effects on meta-analyses whereby a body of literature on a given topic is summarized by transforming test statistics into a standardized metric called effect size. A central tenet of this approach is that the literature under review is unbiased. Song *et al.* (2000) discuss several types of publication bias that can be summarized as submission, review, and editorial bias. Palmer (2000) presents funnel graphs to detect unpublished studies. Unpublished data (submission bias in Song *et al.* 2000) are often those that yielded non-significant results. All of these occur after the original data collection (be it a field study, laboratory, or literature review based approach). However, the types of bias of concern in our study are primarily those that drive our original choice of study system. Nonetheless, it is self-evident that both *a priori* and *a posteriori* biases will result in a literature set that



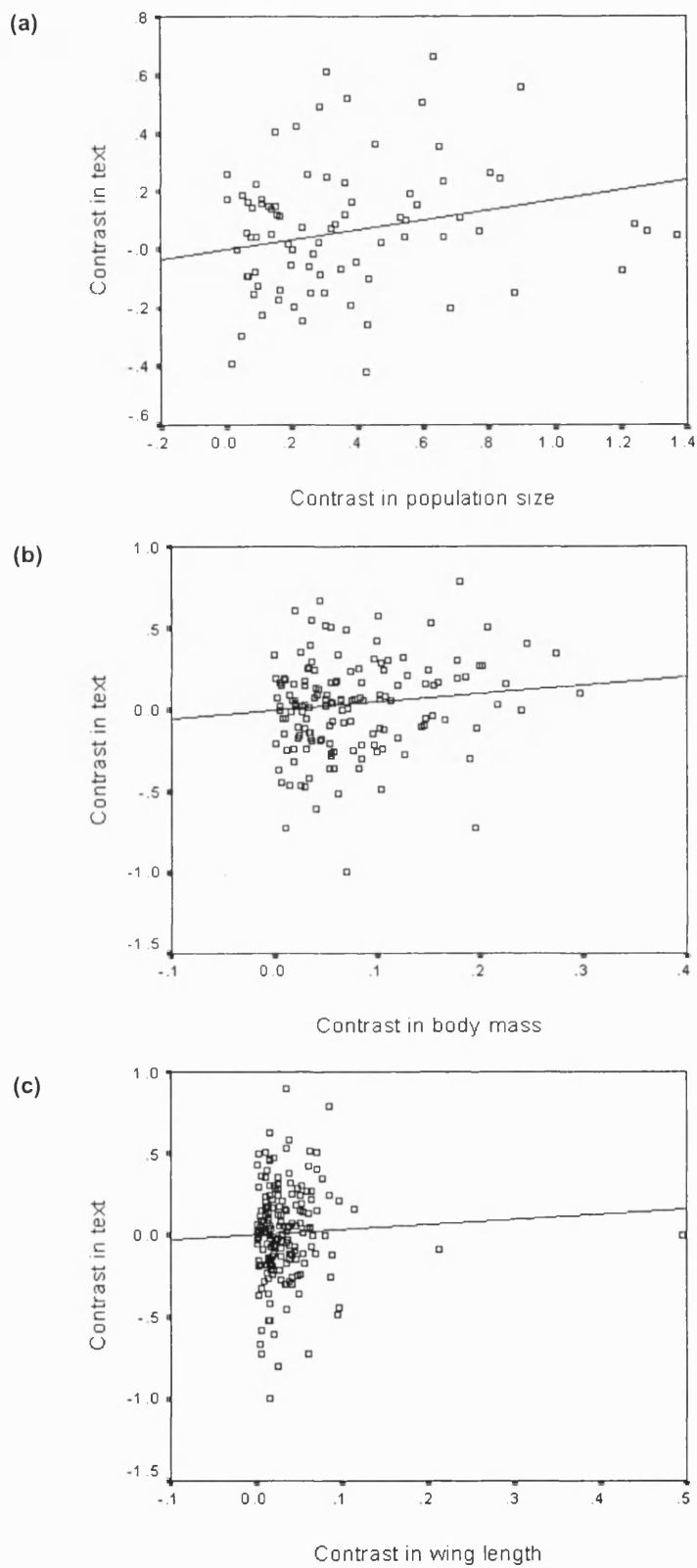


Fig. 2. Regression through the origin between text contrasts and (a) population size contrasts, (b) body mass contrasts, (c) wing length contrasts. All data were  $\log_{10}(x+1)$  transformed prior to calculation of contrasts.



cannot be relied upon as a representative picture of genuine biological trends. For example, if we accept that body size and population size correlate (Gaston & Blackburn 1996) then, based on the findings of the present study, any wader study that looks for trends associated with body size is likely to be biased due to over-representation of smaller taxa (i.e. those which we expect to have larger population sizes). Furthermore, a disturbing conservation issue is revealed. The trend towards studies of taxa with large population sizes suggests that scarce taxa are being overlooked, and species such as the Eskimo curlew (*Numenius borealis*), rated 7 (critical) by BirdLife International (Stattersfield & Capper 2000), have not appeared in the literature according to WoS (since 1980). So not only are the results of any study that seeks to use the literature potentially affected to some degree by publication bias, but conservation efforts may also be impaired by *a priori* selection of study organisms.

## CONCLUSIONS

Our results presented herein suggest that the choice of study taxa amongst wader enthusiasts is governed predominantly by population size, and that northern hemisphere species are better studied than their southern relatives. These are significant because they mean that typically we are severely lacking in important data on the more endangered species and that evolutionary interpretations may be prone to publication bias. Of course, publications are unlikely to represent the full spectrum of research carried out. Many results will go unpublished, and this is perhaps the crux of the problem. All scientists need to publish, and there are numerous ways of increasing publication success. Choosing a taxon or system from which large sample sizes and robust results can be acquired is surely one of them, but there are others. As a cautionary tale, we conclude with quotes from two leading biologists. John Krebs in a talk at Oxford recalled his advice to prospective PhD students (H.P. Sitters, pers. comm.): "Always work on a well-known system. Do that and the world will beat a pathway to your door. Work on something obscure and your thesis will gather dust." An alternative view is provided by Edward Wilson (Seeley 2001): "When choosing a thesis topic, carefully assess where the biggest scientific battles are being waged, where the intellectual action is the hottest, then move as fast as you can in the opposite direction."

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# Appendix 1 – list of wader citations

Species	Number of citations in title-only	Number of citations in text	Species	Number of citations in title-only	Number of citations in text
<i>Syrhaptus tibetanus</i>	0	0	<i>Tringa guttifer</i>	0	0
<i>Syrhaptus paradoxus</i>	1	1	<i>Tringa melanoleuca</i>	3	4
<i>Pterocles alchata</i>	2	3	<i>Tringa flavipes</i>	2	8
<i>Pterocles namaqua</i>	5	13	<i>Tringa solitaria</i>	1	1
<i>Pterocles exustus</i>	0	1	<i>Tringa ochropus</i>	2	3
<i>Pterocles senegallus</i>	0	0	<i>Tringa glareola</i>	2	4
<i>Pterocles gutturalis</i>	1	1	<i>Tringa cinerea</i>	0	0
<i>Pterocles orientalis</i>	2	4	<i>Tringa hypoleucos</i>	1	4
<i>Pterocles coronatus</i>	0	0	<i>Tringa macularia</i>	0	0
<i>Pterocles personatus</i>	1	1	<i>Tringa brevipes</i>	0	0
<i>Pterocles decoratus</i>	0	1	<i>Tringa incana</i>	0	1
<i>Pterocles bicinctus</i>	1	3	<i>Catoptrophorus semipalmatus</i>	9	33
<i>Pterocles quadricinctus</i>	0	0	<i>Prosobonia cancellata</i>	0	1
<i>Pterocles indicus</i>	0	0	<i>Prosobonia leucoptera</i>	0	0
<i>Pterocles lichtensteinii</i>	0	0	<i>Arenaria interpres</i>	13	59
<i>Pterocles burchelli</i>	0	2	<i>Arenaria melanocephala</i>	2	6
<i>Attagis gayi</i>	0	0	<i>Limnodromus griseus</i>	3	13
<i>Attagis malouinus</i>	0	0	<i>Limnodromus scolopaceus</i>	0	10
<i>Thinocorus orbignyianus</i>	0	0	<i>Limnodromus semipalmatus</i>	0	0
<i>Thinocorus rumicivorus</i>	1	3	<i>Aphriza virgata</i>	0	4
<i>Pedionomus torquatus</i>	3	5	<i>Calidris tenuirostris</i>	1	7
<i>Scolopax rusticola</i>	11	13	<i>Calidris canutus</i>	36	132
<i>Scolopax mira</i>	0	0	<i>Calidris alba</i>	15	40
<i>Scolopax saturata</i>	0	0	<i>Calidris pusilla</i>	13	52
<i>Scolopax celebensis</i>	0	0	<i>Calidris mauri</i>	12	53
<i>Scolopax rochussenii</i>	0	0	<i>Calidris minuta</i>	3	8
<i>Scolopax minor</i>	3	21	<i>Calidris ruficollis</i>	1	8
<i>Gallinago solitaria</i>	0	0	<i>Calidris temminckii</i>	3	5
<i>Gallinago hardwickii</i>	2	3	<i>Calidris subminuta</i>	0	0
<i>Gallinago nemoricola</i>	0	0	<i>Calidris minutilla</i>	5	20
<i>Gallinago stenura</i>	1	1	<i>Calidris fuscicollis</i>	6	16
<i>Gallinago megala</i>	0	0	<i>Calidris bairdii</i>	1	1
<i>Gallinago media</i>	11	48	<i>Calidris melanotos</i>	3	11
<i>Gallinago gallinago</i>	6	28	<i>Calidris acuminata</i>	0	2
<i>Gallinago nigripennis</i>	1	1	<i>Calidris maritima</i>	16	24
<i>Gallinago macrodactyla</i>	0	0	<i>Calidris ptilocnemis</i>	1	2
<i>Gallinago paraguayae</i>	0	0	<i>Calidris alpina</i>	58	137
<i>Gallinago andina</i>	0	0	<i>Calidris ferruginea</i>	5	18
<i>Gallinago nobilis</i>	0	0	<i>Micropalama himantopus</i>	2	2
<i>Gallinago undulata</i>	0	0	<i>Tryngites subruficollis</i>	6	11
<i>Gallinago jamesoni</i>	0	0	<i>Eurynorhynchus pygmeus</i>	2	2
<i>Gallinago stricklandii</i>	0	0	<i>Limicola falcinellus</i>	2	5
<i>Gallinago imperialis</i>	0	0	<i>Philomachus pugnax</i>	22	57
<i>Lymnocyrtus minimus</i>	0	0	<i>Steganopus tricolor</i>	1	1
<i>Coenocorypha pusilla</i>	2	2	<i>Phalaropus lobatus</i>	8	22
<i>Coenocorypha aucklandica</i>	2	3	<i>Phalaropus fulicaria</i>	0	6
<i>Limosa limosa</i>	8	29	<i>Rostratula benghalensis</i>	1	2
<i>Limosa haemastica</i>	1	6	<i>Rostratula semicollaris</i>	0	0
<i>Limosa lapponica</i>	9	34	<i>Actophilornis africanus</i>	2	2
<i>Limosa fedoa</i>	0	15	<i>Actophilornis albinucha</i>	0	0
<i>Numenius minutus</i>	1	1	<i>Microparra capensis</i>	0	0
<i>Numenius borealis</i>	0	0	<i>Irediparra gallinacea</i>	2	2
<i>Numenius phaeopus</i>	16	46	<i>Hydrophasianus chirurgus</i>	0	0
<i>Numenius tahitiensis</i>	3	10	<i>Metopidius indicus</i>	2	4
<i>Numenius tenuirostris</i>	2	3	<i>Jacana spinosa</i>	3	7
<i>Numenius arquata</i>	20	43	<i>Jacana jacana</i>	5	11
<i>Numenius americanus</i>	6	19	<i>Chionis alba</i>	1	4
<i>Numenius madagascariensis</i>	2	4	<i>Chionis minor</i>	8	10
<i>Bartramia longicauda</i>	4	11	<i>Pluvianellus socialis</i>	0	0
<i>Tringa erythropus</i>	0	3	<i>Burhinus oedicnemus</i>	13	17
<i>Tringa totanus</i>	29	88	<i>Burhinus senegalensis</i>	0	0
<i>Tringa stagnatilis</i>	0	2	<i>Burhinus vermiculatus</i>	0	0
<i>Tringa nebularia</i>	3	9	<i>Burhinus capensis</i>	1	2



## Appendix 1 cont. – list of wader citations

Species	Number of citations in title-only	Number of citations in text	Species	Number of citations in title-only	Number of citations in text
<i>Burhinus bistratus</i>	1	1	<i>Charadrius asiaticus</i>	0	0
<i>Burhinus superciliaris</i>	1	1	<i>Charadrius veredus</i>	0	1
<i>Burhinus grallarius</i>	0	0	<i>Charadrius montanus</i>	6	12
<i>Burhinus recurvirostris</i>	0	0	<i>Charadrius modestus</i>	0	0
<i>Burhinus giganteus</i>	0	0	<i>Charadrius rubricollis</i>	1	1
<i>Haematopus ostralegus</i>	112	292	<i>Thinornis novaeseelandiae</i>	0	0
<i>Haematopus meadewaldoi</i>	1	1	<i>Erythronyx cinctus</i>	0	1
<i>Haematopus moquini</i>	7	14	<i>Eudromias morinellus</i>	0	0
<i>Haematopus finschi</i>	0	0	<i>Oreopholus ruficollis</i>	0	0
<i>Haematopus bachmani</i>	3	21	<i>Anarhynchus frontalis</i>	0	1
<i>Haematopus palliatus</i>	5	19	<i>Phegornis mitchellii</i>	0	0
<i>Haematopus longirostris</i>	1	2	<i>Peltohyas australis</i>	0	1
<i>Haematopus unicolor</i>	1	2	<i>Elseynornis melanops</i>	0	0
<i>Haematopus fuliginosus</i>	0	1	<i>Vanellus vanellus</i>	51	125
<i>Haematopus ater</i>	1	1	<i>Vanellus crassirostris</i>	0	0
<i>Haematopus leucopodus</i>	0	0	<i>Vanellus malabaricus</i>	0	0
<i>Ibidorhyncha struthersii</i>	2	2	<i>Vanellus macropterus</i>	0	0
<i>Himantopus himantopus</i>	3	7	<i>Vanellus tricolor</i>	0	1
<i>Himantopus leucocephalus</i>	0	1	<i>Vanellus miles</i>	1	3
<i>Himantopus novaeseelandiae</i>	4	7	<i>Vanellus armatus</i>	1	2
<i>Himantopus mexicanus</i>	4	22	<i>Vanellus spinosus</i>	3	6
<i>Himantopus melanurus</i>	2	4	<i>Vanellus duvaucelii</i>	1	1
<i>Cladorhynchus leucocephalus</i>	0	2	<i>Vanellus tectus</i>	0	0
<i>Recurvirostra avosetta</i>	15	28	<i>Vanellus melanocephalus</i>	0	0
<i>Recurvirostra americana</i>	7	40	<i>Vanellus cinereus</i>	0	0
<i>Recurvirostra novaehollandiae</i>	0	0	<i>Vanellus indicus</i>	0	0
<i>Recurvirostra andina</i>	0	0	<i>Vanellus albiceps</i>	0	0
<i>Pluvialis apricaria</i>	16	33	<i>Vanellus senegallus</i>	0	0
<i>Pluvialis fulva</i>	3	14	<i>Vanellus lugubris</i>	0	0
<i>Pluvialis dominica</i>	5	14	<i>Vanellus melanopterus</i>	0	1
<i>Pluvialis squatarola</i>	18	73	<i>Vanellus coronatus</i>	2	6
<i>Charadrius obscurus</i>	1	3	<i>Vanellus superciliosus</i>	0	0
<i>Charadrius hiaticula</i>	5	22	<i>Vanellus gregarius</i>	1	1
<i>Charadrius semipalmatus</i>	1	22	<i>Vanellus leucurus</i>	0	0
<i>Charadrius placidus</i>	0	0	<i>Vanellus cayanus</i>	0	0
<i>Charadrius dubius</i>	3	8	<i>Vanellus chilensis</i>	0	3
<i>Charadrius wilsonia</i>	1	9	<i>Vanellus resplendens</i>	0	0
<i>Charadrius vociferus</i>	6	37	<i>Dromas ardeola</i>	2	3
<i>Charadrius thoracicus</i>	0	0	<i>Pluvianus aegyptius</i>	1	1
<i>Charadrius sanctaehelenae</i>	0	0	<i>Rhinoptilus africanus</i>	0	0
<i>Charadrius pecuarius</i>	0	1	<i>Rhinoptilus chalcopterus</i>	0	0
<i>Charadrius tricollaris</i>	0	0	<i>Rhinoptilus cinctus</i>	0	0
<i>Charadrius forbesi</i>	0	0	<i>Rhinoptilus bitorquatus</i>	1	1
<i>Charadrius melodus</i>	11	53	<i>Cursorius cursor</i>	0	0
<i>Charadrius pallidus</i>	0	0	<i>Cursorius rufus</i>	0	1
<i>Charadrius alexandrinus</i>	10	52	<i>Cursorius temminckii</i>	1	1
<i>Charadrius marginatus</i>	6	10	<i>Cursorius coromandelicus</i>	0	0
<i>Charadrius ruficapillus</i>	1	4	<i>Glareola pratincola</i>	3	6
<i>Charadrius peronii</i>	0	0	<i>Glareola maldivarum</i>	0	0
<i>Charadrius javanicus</i>	0	0	<i>Glareola nordmanni</i>	1	2
<i>Charadrius collaris</i>	1	1	<i>Glareola ocularis</i>	0	0
<i>Charadrius bicinctus</i>	2	6	<i>Glareola nuchalis</i>	0	0
<i>Charadrius alticola</i>	0	0	<i>Glareola cinerea</i>	0	0
<i>Charadrius falklandicus</i>	1	3	<i>Glareola lactea</i>	0	0
<i>Charadrius mongolus</i>	1	4	<i>Stiltia isabella</i>	0	0
<i>Charadrius leschenaultii</i>	0	3			



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Additional

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PhD thesis - appendices  
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